BAD BREATH

Research Perspectives

Second Edition

Editor: Mel Rosenberg

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Editor

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Ramot Publishing – Tel Aviv University
Second Edition

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Ramot Publishing - Tel Aviv University
(Ramot of Tel Aviv University Authority
for Applied Research and Industrial Development Ltd.)
32 Haim Levanon St. P.O. Box 39296,
Tel Aviv 61392, ISRAEL
Telephone: 972-3-6428765; 972-3-6408113
Fax: 972-3-6429865
INTERNET: ramot @ ccsg.tau.ac.il

Bad breath: research perspectives / editor, Mel Rosenberg
Includes Index.
ISBN 965-274-173-6
1. Halitosis 2. Odors 3. Oral health 4. Oral hygiene
5. Bacteria—metabolism. I. Rosenberg, Mel.
617.601—dc20

First published in 1995; Second Edition 1997.

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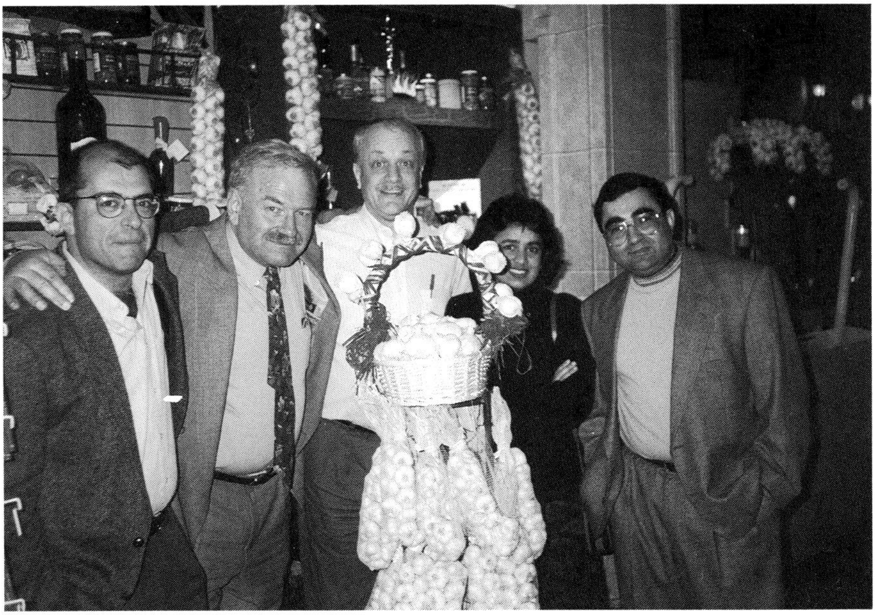
Printed in Israel

Desktop editing: Michal Semo-Kovetz
Cover Design: Tali Niv-Dolinsky

Publication of the first edition of this book was supported by a grant from the Warner-Lambert Company.



Sy Weinstein (second from left) with friends following the UCLA seminar on Bad Breath, March, 1996.

This edition is dedicated to the fond memory of Sy Weinstein.

Contents

Contributors vii

Acknowledgments ix

Foreword to the Second Eddition x

Preface: ***Joseph Tonzetich*** xiii

Chapter 1: Introduction. ***Mel Rosenberg*** 1

Chapter 2: The Biological Basis of Oral Malodor Formation. ***I. Kleinberg and M. Codipilly*** 13

Chapter 3: Strategies to identify the main microbial contributors to oral malodor. ***Walter J. Loesche and Erika H. De Boever*** 41

Chapter 4: Advances in Mouth Odor Research. ***Hollandra P. Niles and Abdul Gaffar*** 55

Chapter 5: Association of Diamines with Oral Malodor. ***Sarit Goldberg, Avital Kozlovsky, and Mel Rosenberg*** 71

Chapter 6: Oral Malodor and Periodontal Disease. ***Ken Yaegaki*** 87

Chapter 7: Relationship of Oral Malodor and Periodontotitis. ***Christopher A.G. McCulloch and Anne Bosy*** 109

Chapter 8: Oral Malodor in the General Population of Japan. ***Hideo Miyazaki, Shigeru Sakao, Yasuhiro Katoh and Tadamichi Takehara*** 119

Chapter 9: Experiences of an Israeli Malodor Clinic. ***Mel Rosenberg and Efrat Leib*** 137

Chapter 10: Non-Oral and Oral Aspects of Oral Malodor. ***G. Preti, H.J. Lawley, C.A. Hormann, B.J. Cowart, R.S. Feldman, L.D. Lowry, and I-M. Young*** 149

Chapter 11: The Otolaryngologist and the Patient with Halitosis. ***Yehuda Finkelstein*** 175

Chapter 12: Halitosis in Patients with Craniofacial Anomalies. ***Yehuda Finkelstein*** 189

Chapter 13: Psychological Factors in Self Assessment of Oral Malodor. ***Ilana Eli, Roni Baht, and Mel Rosenberg*** 201

Appendix 1: Conference Report: First International Workshop on Oral Malodor. ***Mel Rosenberg*** 215

Appendix 2: In Vitro Oral Malodor Assessment. ***Dan Markovich, Sarit Goldberg, Ilana Eli and Mel Rosenberg*** 223

Appendix 3: Current and Future Semiconductor-Based Bad Breath Detectors. ***Ken Yaegaki*** 227

Appendix 4: BDA Fact File 232

Index 237

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Acknowledgments

First Edition

The present text grew out of the First International Workshop on Oral Malodor, held in Israel on April 26th-28th, 1993. However, **Bad Breath: Research Perspectives** is not a Proceedings in the strict sense since the chapters were written over a year following the meeting, enabling authors to include new research findings and insights. Since not all the presentations at the Workshop led to chapters in the book, I have included a report of the Workshop which was published recently in the Journal of Dental Research (73:586-589, 1994) and is reprinted here with permission. Neither the Workshop nor the present book would have been possible without the generous support of the Warner-Lambert Company.

Everyone in the field of oral malodor research is indebted to Dr. Joseph Tonzetich, of the University of British Columbia. Dr. Tonzetich deserves the credit for almost singlehandedly turning bad breath into a science. I am particularly grateful that he was able to contribute the Preface of this text.

I am indebted to Rita Lazar for her excellent editorial work in review and preparation of the manuscripts, to Michal Semo-Kovetz who singlehandedly prepared the text for publication, and to Tali Niv-Dolinsky for the outstanding cover design. Thanks are also due to Hani Tal, Sarit Goldberg and Orit Ilan for their meticulous reading of the manuscripts. I am extremely grateful to Sy Weinstein, Warner-Lambert Company for his advice throughout both the organization of the Workshop and preparation of the book. Special thanks go to my wife, Shuli, for her help in organizing the Workshop, and for her support and counsel through the years.

My own interest in the diagnosis and treatment of bad breath was kindled over the past decade by many friends and colleagues who provided advice and encouragement. In particular, I am grateful to Bruce Baum, Ronit Bar Ness-Greenstein, Benjamin Barish, Zvi Ben Shoshan, William H. Bowen, Dan Deutsch, R.J. Doyle, Ilana Eli, Richard P. Ellen, Alison Fleming, Oded Gasko, Shimon Goldstein, Dan Inbar, Israel Kleinberg, Yoel Konis, Avital Kozlovsky, Karl Laden, Norman Levine, Dan Malamud, Chaim Regev, Yoav Sadeh, Smadar Shilo, Barry J. Sessle, Calvin Torneck, Ervin I.Weiss, Pinni Yaniv, and Mal Yasny for their support. I am also grateful to the colleagues with whom I have been privileged to collaborate on this subject, with special thanks to Christopher A.G. McCulloch and Jacob Gabbay. Finally, I thank all the contributing authors for their excellent chapters.

Bad Breath: Research Perspectives is intended to reflect a range of different approaches to the study of bad breath. The opinions expressed by the various authors are their own (and at times, not altogether in concert with those of the editor or one another). I take complete responsibility for omissions and errors, hoping that there are not two many of either.

Mel Rosenberg
Tel Aviv, February, 1995

Foreword to the Second Edition

This second edition of **Bad Breath: Research Perspectives** is dedicated to the memory of our dear friend and colleague, Sy Weinstein, who passed away suddenly on May 31st, 1996. To everyone who knew him, Sy was a source of inspiration, encouragement and optimism. During the past five years, Sy's interest in the field of oral malodor led to organization of the first international conference dedicated to the subject, which in turn led to publication of the first edition of this text in 1995. Based on Sy's recommendation and support, both of these enterprises were sponsored by Warner-Lambert Company, where Sy served as Director of New Product Concepts.

When **Bad Breath: Research Perspectives** was first published, we never anticipated that within two years a second edition would be underway. The book was favorably reviewed in five journals, including the Journal of Dental Research and the Journal of the American Dental Association. Based on the suggestion of one reviewer (Dr. McDowell), Ken Yaegaki agreed to present an update on commercial bad breath detectors, which appears as Appendix III in the new edition. Other suggestions which have been incorporated in the second edition include higher quality reproductions in Chapters 11 and 12 and correction of several typographical errors. We have also reproduced, with permission of the British Dental Association, a **Fact File** on bad breath which is currently distributed in the United Kingdom (Appendix IV).

During the past couple of years, interest in the diagnosis and treatment of bad breath has continued to grow, with thousands of dentists currently offering breath freshening as part of their dental practice. The Second International Workshop on Oral Malodor, held in Leuven, Belgium, in Ocober, 1995, was a great success, with some 140 participants from 16 countries. At the meeting, the establishment of the "International Society for Breath Odor Research" was conceived. The Society is now an entity, with its own **Newsletter**. The proceedings of the 1995 Workshop, "**Bad Breath: A Multidisciplinary Approach**", (D. van Steenberghe and M. Rosenberg, ed., Catholic University Leuven Press) has just appeared. Much of the credit for organization of the Second Workshop and publication of the Proceedings goes to Daniel van Steenberghe, whose interest and dedication have largely contributed to the continuity and growth of this research field. As we go to press, Donald M. Brunette and Joseph Tonzetich are hard at work organizing the **Third International Conference on Breath Odour**, to be held August 22-23, 1997, in Vancouver, Canada, with an expected attendance of some 400 researchers and interested professionals.

Preface

Joseph Tonzetich[1]

The scientific approach to studying halitosis, commonly referred to as bad breath, is attracting increasing interest in a broad spectrum of diverse disciplines, especially in the dental and medical fields. The resulting accelerated accrual of scientific knowledge dictates periodic meetings, such as the First International Workshop on Oral Malodor, and a collation of the proceedings, as exemplified by this book, is intended to portray the current state of knowledge and generate ideas for future directions of research.

Prior to the late 1930s, most citations pertaining to halitosis consisted mainly of anecdotal statements that have been perpetuated in the literature. Subsequently a number of notable developments have elevated the field to a sound scientific level. During the 1940s and early 1950s, Fosdick and his associates at Northeastern University, through the use of an osmoscope, an instrument designed and developed by Fair and Wells in 1934 for subjective semi-quantitative measurement of odor intensity, conducted numerous studies that produced valuable information on sources and conditions contributing to malodor emitted via the mouth.

They found that while malodor may be due to physiological and/or

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pathological causes of oral, systemic or nasopharyngeal origin, the principal cause of halitosis is of physiological origin involving the oral cavity. Recent studies implicate the dorso-posterior surface of the tongue as the principal source. However, in individuals with periodontal disease, the contribution from the gingival crevice increases with the severity of involvement. In advanced cases, significant amounts of the malodorous components, CH_3SH predominating, are derived from the diseased sites.

Halitosis is one of the most frequent complaints voiced by dental patients. It merits concern as virtually all individuals may occasionally experience episodes of malodor. Furthermore, approximately 50% of middle-aged and older individuals emit socially unacceptable breath, attributed to physiological causes, upon arising in the morning. While this transient condition is readily controlled, persistent halitosis may be indicative of disorders, such as gastro-intestinal ulcers, internal hemorrhage, hiatus hernia, diabetes mellitus, hepatic cirrhosis, leukemia, uremia, and many non-characterized idiopathic conditions. The malodor in these disorders is persistent, more intense, usually characteristic of the condition, and difficult to control. Hence, the importance of persistent malodor cannot be overstated as it may be indicative of an underlying pathology. Superimposition of pathological malodor on physiological malodor may make early morning breath extremely offensive.

Yet, in spite of the apparent general concern and pathological implications of halitosis, health professionals, including dentists, lack adequate knowledge of the subject to properly counsel patients. Hence, this and future workshops on malodor should not only be of value to research, but fulfill an important function of disseminating information to scientists, health professionals, and the population at large.

During the 1960s and 1970s the emphasis of oral malodor research focused on applications of chemical and instrumental methods of analysis aimed at identification of primary malodorous compounds. On the basis of this information, a highly sensitive and specific gas chromatographic (GC) method was adapted for direct measurement of volatile sulfur compounds (VSC) content of headspace of saliva and mouth air.

Initial studies were performed on headspace of saliva. Through the addition of substrate and enzyme inhibitors to saliva, it was established that VSC are the principal offending substances responsible for the malodor of putrefied saliva. While coulometric (Titrilog) and mass spectrometric (MS) analyses were found acceptable for headspace analysis, they were unsuitable for direct measurement of VSC in mouth air. The Titrilog was insufficiently sensitive while only tentative identification of H_2S, CH_3SH and dimethyl disulfide was possible with MS. Significantly, the presence of mono- and diamines was not detected by any of the reported chemical, MS and GC direct analyses of headspace vapor or mouth air.

Initial GC investigations employing units with flame-ionization detectors were only of a limited value in detecting the presence of VSC in mouth air. It was not until the advent of a GC equipped with a highly sensitive and selective flame-photometric detector (FPD) that measured only sulfur-containing compounds but ignored others, that it was possible to identify and permit direct measurement of individual VSC components (H_2S, CH_3SH, and $[CH_3]_2S$) in mouth air. The H_2S and CH_3SH, which comprise approximately 90% of the VSC content, are imputed to be the major cause of physiological putrefactive oral malodor. Since 1970, the GC/FPD unit has been the instrument of choice for research and clinical studies. It has been particularly applicable for determining the odor-inhibiting capacity of dental products, as the masking effect of flavors do not interfere with the analysis. The masking effect in these trials can also be conveniently determined by employing a combination of the organoleptic and instrumental methods of analysis.

Subsequently commercially available, compact and inexpensive mercaptan and sulfide monitors (Halimeter) appeared on the scene. It is imperative to validate their performance with respect to the GC/FPD system before their acceptance for measurement of odor intensity. As they lack specificity, their utility is limited and could best be exploited for screening purposes. At this conference, Yaegaki disclosed the development of a prototype bad breath detector which he claims possesses the capability of measuring odor intensity equivalent to that of the GC/FPD system. When available, its applicability will also require critical assessment. The evolution of a portable class of compact monitors is a notable progress in technology as it addresses the need for units suitable for use in field clinical trials and dental/medical offices.

Recently a new chemiluminescence detector (SCD), specific for detection of sulfur, became commercially available. It is claimed to have the best selectivity and sensitivity for measurement of low levels of sulfur compounds of all commercially-available sulfur-selective detectors, including FPD. The flame-based unit is claimed to be more sensitive than FPD by at least a factor of 10, and the flameless unit is approximately two orders of magnitude more sensitive than FPD.

Aside from the malodor consideration of H_2S and CH_3SH, a large body of evidence has accumulated during the past ten years which suggests that they are also of major pathological importance. Through non-keratinized mucosal tissue, tonsillar mononuclear and gingival fibroblast cell culture studies, it was found that volatile thiol compounds, especially CH_3SH, exert a number of adverse effects on the structure and metabolism of oral tissues. These effects are noteworthy as they assign a role for volatile thiols in the etiology of periodontal disease.

In vitro permeability studies conducted on non-keratinized oral mucosa reacted with $[^{35}S]$-labelled VSC have shown that H_2S and CH_3SH can penetrate through, and react with, all three tissue layers (epithelium, basal membrane and connective tissue) of the mucosa. In the process they markedly increase the permeability of the exposed mucosa to ions (SO_4^{-2}), molecules (PGE_2) and to a lesser extent bacterial lipopolysaccharides (LPS). While the H_2S-treated mucosa can be readily restored to a normal state, the reversal of the CH_3SH effect is considerably more difficult to achieve.

The scanning electron microscopic examination of the CH_3SH-exposed epithelial surface of fresh mucosal tissues shows a markedly rough surface indicative of cell exfoliation and dissolution of intercellular matrix. The transmission electron microscopic inspection of these specimens shows drastic disruption and extensive obliteration of the basal membrane, while the connective tissue layer displays evidence of vacuolation and general decrease and thinning of collagen fibers. The observed changes in the basal membrane and connective tissue layers are supported by antibody studies against fibronectin, laminin and collagen.

Studies on $[^{35}S]$-H_2S treated types I and III acid-soluble collagens demonstrate that the volatile thiols can also exert a detrimental effect on the integrity of collagen. The binding of $[^{35}S]$-activity occurs through at least two active sites on the collagen molecule. CM-cellulose chromatography and SDS-PAGE electrophoresis analyses indicate that the bonds are quite stable as a measurable amount of $[^{35}S]$ activity was still associated with α and β peptide chains after exposure to chemical agents and required analytical manipulations.

Furthermore, studies employing human gingival fibroblast cultures have revealed that both thiols can adversely affect cellular metabolism. They can significantly reduce proline transport and DNA, collagen and non-collagenous protein synthesis. They also increase the intracellular and extracellular rate of collagen degradation, especially the extracellular component where over 50% of the synthesized collagen is found in a degraded state. The combined effect of decreased synthesis and increased degradation in thiol-exposed culture results in an approximately 70% lower collagen content, compared with control systems.

Significantly, collagen loss of the same magnitude has been found in periodontally-involved gingival connective tissue. This observation is supported by ELISA analysis findings which show significantly increased amounts of collagen-derived components in crevicular fluid collected from diseased tissue sites. It has also been reported that CH_3SH lowers the resting cytosolic pH and inhibits its rate of recovery in fibroblasts.

Neutral proteases, especially collagenase, are believed to play a major role in the degradation of collagen observed in periodontitis. The increase in mammalian collagenase is reputed to occur through activation of the immune response system triggered by LPS stimulation of mononuclear cells to produce increased amounts of IL-1. This initiates a cascade of events which ultimately result in increased neutral protease production and collagen destruction.

As 2-mercaptoethanol is normally used experimentally to augment the LPS activation of IL-1 production, it is not surprising that CH_3SH was shown to increase the stimulation index of LPS-exposed tonsillar mononuclear cell cultures by 2-fold. Furthermore, CH_3SH alone, or in combination with IL-1 or LPS, significantly enhances the secretion of PGE_2, cAMP and procollagenase by human gingival fibroblast cultures. In view of the described findings, the following pathway is proposed whereby CH_3SH may increase collagen degradation through the immune response system.

The bacterial endotoxins (LPS) and CH_3SH activate the macrophages to produce IL-1 which can (i) act directly on T-lymphocytes to cause amplification of hypersensitivity reactions resulting in tissue destruction; or (ii) exert an effect alone, or in synergism with CH_3SH on fibroblasts to produce increased levels of PGE_2 and cAMP. This results in stimulation and increased production of active neutral proteases, such as plasmin, collagenase, elastase and thiol-dependent cathepsin B, which in turn leads to increased destruction of collagen. These investigations have opened a window to a virtually unexplored field of how the thiol by-products of metabolism may affect the progression of periodontal disease and other degenerative disorders, especially those affecting connective tissues.

Judging from the caliber of research presented by the participants at the First International Workshop, oral malodor research is attracting many well-qualified investigators. In their critical review of research accomplishments to date, they identified some significant areas of research that require investigation. Besides their succinct suggestions on directions of future malodor research, recently reported by Rosenberg (see Appendix 1), there are a number of other broader areas of concern, especially those related to the pathological aspects of oral malodor.

At the present time no other analytical technique equals the exceptional capability of GC for detection and measurement of malodorous compounds. However, with its present capability, only approximately 10 different compounds can be analyzed by direct mouth air and breath sampling techniques. As enrichment techniques indicate that there are more than 100 compounds present in trace ppb amounts in breath, there is a pressing need to develop more sensitive and specific detectors. The continued progress in technology, as exemplified by the introduction of the SCD sulfur-specific detector, which requires critical assessment and comparison to the GC/FPD system for mouth air analysis, suggests that this is a reasonable expectation. The improved sensitivity of SCD would enable measurement of longer aliphatic chain volatile thiols and sulfides, such as dimethyl disulfide which has been detected by FPD in enriched, and occasionally in unmanipulated, mouth air samples. The ability to detect trace amounts of other VSC may be useful in correlating their presence with specific diseases and their impact on the quality and intensity of malodor.

There is also a critical need to attain a similar level of analytical sophistication for analysis of other compounds, especially the amines. Numerous studies and literature reviews since 1940 have advocated, on the basis of presumptive evidence, that amines, formed through salivary putrefaction and capable of being purged under non-physiological conditions, are important components of oral malodor. This supposition is not tenable as their presence has not been detected in any of the reported analytical studies conducted directly on mouth air. Hence it is essential to develop a sensitive and selective N-detector capable of detecting amines, if present, at concentrations found in mouth air. If their concentrations are established, then their direct impact on oral malodor can be more objectively appraised. There is also the possibility that the presence of amines in putrefied saliva may indirectly enhance odor by mechanisms similar to the involvement of putrescine in enhancement of collagenase production by monocytes/macrophages.

The emerging technology devoted to development of easily operated compact instruments, such as the Halimeter and CH_3SH-detector units adaptable for dental/medical office and in-home use requires further refinement. In addition to sampling problems with CH_3SH-detector units, the chief drawbacks of both of these units in their present form is their lack of specificity and inability to discern the types and concentrations of individual components in test samples. This limits their applicability for research purposes. However, further improvement of sampling techniques and development of more selective detectors adaptable to these compact units would make them extremely attractive for use in clinical trials.

Information is required as to which compounds, or class of compounds, though non-malodorous themselves or occurring below the threshold of organoleptic perception, can alter the intensity and quality of malodor. This relatively ignored area of research could provide an insight on characteristic malodors associated with specific conditions, such as a sour-type odor emitted by aged individuals, a very distinctly different odor emitted by ulcer patients, and odor associated with hepatic cirrhosis patients that has been described as ammoniacal, but later attributed to CH_3SH and dimethyl disulfide. In these three examples, and others, the VSC are the principal cause of malodor, but in each case the malodor is distinctly different. While this may in part be attributed to differences in mixtures and ratios of S-compounds, there is a strong possibility that non-sulfur compounds may also be involved.

The demonstrated detrimental effect of volatile thiols in tissue and cell culture studies presents a vast fertile area for future biochemical research. Of immediate concern is whether the observed general suppression of protein synthesis, including collagen, occurs at the level of m-RNA gene expression or at some other stage of the pathway. More detailed information is also required on their effects on proteolysis and other intra- and extracellular biochemical processes. These studies should be expanded to include other products of salivary putrefaction, including putrescine and cadaverine.

The studies to date suggest that CH_3SH may be a contributing factor in the enzymatic and immunologic events leading to tissue degeneration in periodontal disease. It has been demonstrated that it assists in stimulation of mononuclear cells to produce the pyrogenic cytokine IL-1. In the context of the pathogenesis of periodontal disease, this initiates a cascade of events that result in increased degeneration of the periodontal tissues. Moreover, CH_3SH may also act via a second pathway. If CH_3SH can directly stimulate the production of TNFα and IFNγ or TGFβ, the pattern of integrin expression on epithelial and fibroblast derived cells may be altered. Integrins, the cell surface glycoproteins that mediate cell-cell and cell-extracellular matrix adhesion are cysteine-rich molecules. If exposed, these thiol and disulfide groups may react with CH_3SH and thereby alter the transmission of molecular signals that regulate cell activity.

It is hoped that this account of oral malodor research conveys the realization that it is a growing science that extends far beyond the social aspects of malodor and that it has an immense potential to expand our knowledge of cell function in normal and disease states.

1

Introduction

Mel Rosenberg

Bad breath is a problem that traverses history, culture, race and sex. Writings on oral malodor date back to Greek and Roman times (see, for example Prinz, 1930; Geist, 1957; Spouge, 1964; Mandel, 1988). Jewish liturgical teachings, dating back almost two millennia, state that a man who marries a woman, and subsequently discovers that she has bad breath, can summarily divorce her without fulfilling the terms of the marriage contract (ketuba). Islamic theology stresses the importance of the Siwak (a special stick for cleaning the mouth), including its use during the fast of Ramadan to prevent bad breath. The prophet Muhamed is said to have expelled a man who had previously eaten garlic from the mosque (S. Orenbuch; I. Natour; personal communications).

Interestingly, the only previous English-language treatise on bad breath was written in the last century and was apparently well received, having been reprinted at least three times (Howe, 1898). The present book, being the first multi-authored text to focus on bad breath, is intended to reflect the state-of-the-art, as expressed by world leaders in this field. Clearly, this is an area in which public interest still outstrips scientific endeavor. We can only hope that in coming years a growing cadre of talented scientists will

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undertake to move this field forward.

Contrary to some folk beliefs, bad breath usually originates in the mouth itself and is generally amenable to treatment. How unfortunate, then, that so many people remain afflicted with this condition, and are often unaware that they even suffer from it. I recall the story of a man who, in his youth, was in love with a pretty young lady, but because of her oral malodor, ended up marrying another. Thirty years later, after hearing a lecture on the subject of bad breath, he confided that had he heard the lecture as a bachelor, he might have wound up marrying his first love.

Few human characteristics remain as hard to detect in oneself as bad breath. Should we want to hear how we sound, we can play back an audio recording. If we wish to see how we look, talk and act, we can watch ourselves on video. People appear to be unaware of their own breath, yet are able to detect it in others. Early reviewers (see, for example, Spouge, 1964) blamed the inability to smell our own breath on adaptation (*i.e.*, that one's sense of smell is dulled by continuous exposure). In a recent study, people were asked to score the level of malodor emanating from their mouth, licked wrist and expectorated saliva (Rosenberg *et al.*, 1995). The results suggest that preconceived notions of how much bad breath one thinks one *should* have has a major confounding effect on subsequent scoring of self-measurements. Our reluctance to notify even those close to us that they suffer from this condition serves to further exacerbate the situation. Thus, many people suffer unnecessarily from bad breath throughout their lives, without being remotely aware of it.

Measurement of Bad Breath

Modern day scientists prefer to measure when they can, and to describe what they cannot measure. To some extent, bad breath defies both measurement and verbal description. The characteristics of a given smell can be likened to another ("smells like fried onions"), but smells cannot be described beyond very general terms, such as fragrant, noxious, etc. The "gold standard" of bad breath measurement remains the human nose. However, whereas there is often a consensus in social circles about who has bad breath and who does not, correlations between odor judges in clinical settings are often moderate at best (Rosenberg *et al.*, 1991a,b; Rosenberg and McCulloch, 1992). There is some disagreement over the need to train judges to score bad breath. In the case of known and reproducible odorants, training may be useful. Similarly, training is important if the judges are to make qualitative judgments on odor notes (Engen, 1982). However, in the case of bad breath, it is not clear whether training improves one's ability to score malodor intensity (Rosenberg and McCulloch, 1992). As part of a laboratory course in oral microbiology, 21 undergraduate dental students (prior to their clinical training) at Tel Aviv University were recently asked to rate each other's oral malodor on a 0-5 scale (Rosenberg *et al.*, 1991 a,b), alongside scores given by an odor judge with previous experience (MR). Results are presented in Table 1 and Figure 1. In almost all instances (19 of 21), the students' correlations with the mean scores were significant, and in four cases, exceeded the correlation between the experienced odor judge and class mean.

During the last century, various attempts have been made to develop instrumental approaches to measuring bad breath. These include the osmoscope (Sulser *et al.*, 1939), gas chromatography (Tonzetich, 1977; Schmidt *et al.*, 1978) and, more recently, the use of portable gas monitors. Table 2 summarizes recent correlations obtained between odor judges and a portable sulfide monitor (Interscan Corp., Chatsworth CA). As can be seen, although the correlations themselves are significant, the variation in sulfide levels can account for only some 20-40% of the variation in odor judge scores. Correlations comparing organoleptic measurements with levels of volatile sulfur compounds (VSC), using gas chromatography, can also be low (Schmidt *et al.*, 1978). One possible reason for this is that VSC are not the only gaseous components involved in malodor. Several studies on bad breath have implicated a range of non-sulfur compounds (Kostelc *et al.*, 1980; 1981; see Goldberg *et al.*, this volume). In 1967, Tonzetich *et al.* showed that several of these compounds, *i.e.*, indole, methylamine and cadaverine are not volatilized from the bulk aqueous phase of saliva. However, as shown by Kleinberg and Codipilly (this volume), when such compounds are allowed to dry out on the skin, the odors are released, some over long periods of time. Goldberg *et al.* (1994) have recently shown that cadaverine levels in saliva are associated with odor judge scores and improve the correlation obtained with sulfides alone. Whereas cadaverine measurement may prove an important adjunct measurement to sulfide monitoring, it is not amenable for use in the dental clinic. In this case, the BANA assay may prove useful. This simple color test records the presence of trypsin-like enzymatic activity associated

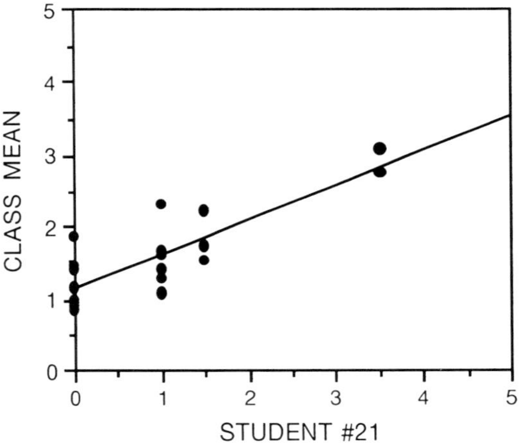

Figure 1. Comparison of the organoleptic scores given by student #21 with the mean scores of the class.

with several putative periodontopathogenic oral species. BANA scores are associated with cadaverine levels and can improve the correlation between sulfide measurements and oral judge scores (De Boever *et al.,* 1994; Goldberg *et al.,* 1994; Kozlovsky *et al.,* 1994a; Loesche and De Boever, this volume). Future quantitative assessments of oral malodor may include electronic "noses" which will provide information on both the intensity, character and source of the offensive smells (Yaegaki, this volume). However, until this occurs, clinical evaluation by instrumental measurement alone is to be discouraged.

Microorganisms and Oral Malodor

It has long been acknowledged that most cases of bad breath are the result of microbial putrefaction within the oral cavity (Howe, 1898; Berg and Fosdick, 1946; McNamara *et al.,* 1972). Many oral bacteria produce hydrogen sulfide and other volatiles when incubated anaerobically in the presence of serum, saliva, or other substrates (Persson *et al.,* 1989; 1990). Indeed, humans classify practically all bacterial odors as foul, perhaps as an age-old mechanism

Table 1
Pearson Correlation Coefficients Comparing Each Student's Scores with the Class Meana

Judge no.	**N**	**Correlation coefficient (r=)**	**Probability (p)**
1	20	0.26	>0.05
2	20	0.29	>0.05
3	20	0.40	<0.05
4	20	0.40	<0.05
5	20	0.41	<0.05
6	20	0.42	<0.05
7	20	0.49	<0.025
8	19	0.50	<0.025
9	20	0.57	<0.01
10	20	0.60	<0.01
11	20	0.61	<0.0025
12	20	0.63	<0.0025
13	19	0.65	<0.0025
14	20	0.65	<0.0025
15	19	0.66	<0.0025
16	20	0.66	<0.0025
17	20	0.71	<0.0005
18	20	0.73	<0.0005
19	20	0.76	<0.0005
20	20	0.77	<0.0005
21	20	0.81	<0.0005
MR[b]	21	0.72	<0.0005

[a] Undergraduate dental students (N=21) were asked to smell and score each other's whole mouth malodor on a semi-integer scale of 0-5, with two minute pauses between measurements (Rosenberg *et al.*, 1991b). Pearson linear regression analysis was carried out comparing each student's set of evaluations with the mean class scores.

[b] Scores (N=21) were concomitantly carried out by an experienced judge of oral malodor (MR).

Table 2
Correlations Between Sulfide Levels Measured Using the Interscan Monitor and Odor Judge Assessments

Subjects	Organoleptic measurement	Correlation coefficient (r=)	Probability (p≤)	Reference
75 Israeli adults	Average of 7 judges	0.603 (Sp[a]) 0.640 (Pe[b])	0.001 0.001	Rosenberg *et al.*, 1991b
41 Canadian adults	2 judges	0.552 (Pe) 0.425 (Pe)	0.001 0.001	Rosenberg *et al.*, 1991a
60 Israeli dental students	1 judge	0.595 (Sp)	0.001	Rosenberg *et al.*, 1992
107 Canadian adults	1 judge	0.525 (Pe)	0.0001	Bosy *et al.*, 1994
55 American adults	1 judge	0.634 (Pe)	0.001	De Boever *et al.*, 1994
52 Israeli adults	1 judge	0.472 (Pe)	0.001	Kozlovsky *et al.*, 1994a

[a] Spearman correlation coefficient
[b] Pearson correlation coefficient

to avoid infections and spoiled food. In their respective chapters, Niles and Gaffar, Kleinberg and Codipilly, and Loesche and De Boever describe ongoing work to identify specific species which may be major contributors to oral malodor *in situ*. Goldberg and coworkers (this volume) used a decarboxylase medium to obtain *in vitro* samples that smell similar to bad breath (see also Markovich *et al.*, this volume). However, attempts to isolate individual strains which produce this odor have not yet proven successful.

Even within the oral cavity, odors from different sites have different characteristic smells. For example, the odor emanating from the posterior tongue dorsum differs greatly from the odor characteristic of subgingival plaque (Rosenberg and Leib, this volume). Future research is necessary to determine whether the distinctiveness derives from different molecular constituents, different ratios of given components *e.g.*, hydrogen sulfide and methyl mercaptan (Yaegaki, this volume), or a combination of both these possibilities. Similarly, it is not known whether these variations in odor are associated with distinct microbial communities and/or availability of different putrefiable substrates.

Oral Malodor and Periodontal Diseases

The relationship between periodontal diseases and oral malodor is a perplexing one. Given the insidious nature of periodontal disease progression, early detection based on oral malodor would seem an ideal tool (McCulloch and Bosy, this volume). Dental professionals encounter the putrid odor of subgingival plaque on a day-to-day basis. Earlier studies (*e.g.*, Sulser *et al.*, 1939; Berg and Fosdick, 1946), as well as more recent ones carried out in Israel (Kozlovsky *et al.*, 1994a), Japan (Miyazaki *et al.*, this volume; Yaegaki and Sanada, 1992; Yaegaki, this volume) and the US (De Boever *et al.*, 1994), show highly significant associations between periodontal disease and oral malodor parameters. However, the results of a recent Canadian study involving a large population, did not support an association between these two parameters (Bosy *et al.*, 1994; McCulloch and Bosy, this volume). Similarly, whereas plaque index appears to correlate with oral malodor in several studies (Rosenberg *et al.*, 1991a; Kozlovsky *et al.*, 1994a), the two are not always associated (Bosy *et al.*, 1994). Future research is required to unravel these apparent paradoxes.

Selection of Subject Populations for Clinical Studies

An interesting aspect of bad breath research involves selection of subject populations. Some studies have excluded women, based on research showing that oral malodor may change as a function of the menstrual cycle (Tonzetich *et al.*, 1978). Since the majority of individuals complaining of oral malodor are women (Rosenberg and Leib, this volume), their participation in clinical studies is warranted. Another question involves mode of recruitment of subjects for oral malodor studies. Based on the data of Eli *et al.* (this volume), subjects who volunteered for a clinical study in response to a newspaper article tended to have altered psychopathological profiles, compared with a general population of dental patients. Thus, investigations employing a wide range of subjects who do not necessarily complain of oral malodor (*e.g.*, Miyazaki *et al.*, this volume) are necessary in order to obtain data more representative of the population as a whole.

Malodor of Non-Oral Etiologies

The nasal passages constitute, in my opinion, the second most frequent source of bad breath, after the mouth itself (Finkelstein, Chapter 11, this volume). Frank nasal odor may lead to discovery of sinus and other infections, obstructions, foreign bodies, etc., but, interestingly, many instances are often unaccompanied by pathological findings. Furthermore, the nasal passages may have a much greater role in oral malodor production than has been previously imagined. Many foul smelling samples scraped from the posterior part of the tongue dorsum bear a physical resemblance to nasal mucus (Rosenberg and Leib, this volume). Could postnasal drip thus constitute a major source of oral malodor? Another area of uncertainty involves the potential role of tonsils in bad breath (Finkelstein, Chapter 11, this volume). Patients with craniofacial anomalies (*e.g.*, cleft palate) may be prone to oral and nasal malodor (Finkelstein, Chapter 12, this volume). I would not be surprised if rhinoplasty also predisposes individuals to bad breath. Hopefully, oral malodor research clinics, such as that recently established in Leuven, Belgium, will shed light on these and other issues. Although many systemic diseases may lead to bad breath (Attia and Marshall, 1982), they account for only a small fraction of cases. The work underway in the laboratory of George Preti and coworkers (this volume) may lead to recognition of the metabolic disorder, trimethylaminuria, as a more significant contributor to perceived or real oral malodor than previously recognized.

Industrial Research on Bad Breath

A glimpse of industry-based research on oral malodor is provided here in the chapter by Niles and Gaffar. During the First International Workshop on Oral Malodor last year, an interesting dichotomy arose between university and industry researchers (see Appendix 1). Whereas the former tend to look at oral malodor as a condition of potential medical importance, industry investigations tend to concentrate on the cosmetic side, *i.e.*, masking of odors of pizza, smoking, etc. As opposed to anti-plaque and anti-gingivitis claims, breath freshening remains a cosmetic claim in many countries. Since water also reduces bad breath for a given period (albeit a very brief one), practically all oral products can fit neatly into this category. Consumers and dentists themselves have a conspicuous difficulty in determining which products actually "take your breath away", and for how long. Few papers have been published on malodor reduction for periods of above 2 or 3 hours. Mouthwashes shown to reduce bad breath for 8 hours or more include 0.2% chlorhexidine formulations (Rosenberg *et al.*, 1991a,b; 1992), Listerine™ (Kozlovsky *et al.*, 1994b) and a recently developed two-phase oil-water mouthwash (Rosenberg *et al.*, 1992; Kozlovsky *et al.*, 1996).

Imaginary Bad Breath ("Halitophobia")

Several of the contributors in this present volume (Eli *et al.*, Miyazaki *et al.*, Rosenberg and Leib, Preti *et al.*, Yaegaki) allude to the problems associated with imaginary bad breath ("halitophobia"). Although various psychological states have been used to describe this population, (*e.g.*, delusion, hypochondria, obsessive-compulsive disorder, olfactory reference syndrome) none are satisfactory. In milder cases, "halitophobics" worry about having bad breath constantly and tend towards obsessive oral care and masking techniques, yet get on with their lives. Others may go from specialist to specialist (Hawkins, 1987), spend decades in isolation, have their teeth extracted (Massler *et al.*, 1951) and may even commit suicide (Yaegaki, this volume). Since the current success rate in dealing with such individuals appears to be low, we must seek ways to develop treatment regimens combining psychological and/or psychiatric counseling together with physiological feedback in order to adequately treat those suffering from this debilitating problem.

Present and Future

Bad breath is a foul odor detected on the breath exiting from the mouth, nose or both. In most cases, bad breath derives from the oral cavity itself and is often amenable to treatment (Tonzetich, 1977). Proper professional treatment and oral hygiene (including deep tongue brushing) with the optional use of efficacious mouthrinsing usually bring about dramatic reductions in oral malodor (Morris and Read, 1949; Scully *et al.*, 1994). In some cases, bad breath presents an important clue that a medical problem exists. The major problem remains one of diagnosis, both in clinical research and individual counseling. How can bad breath best be measured and detected? Is the odor real or imagined? What are the sites (whether in the mouth or elsewhere) or diseases responsible for the presenting odor? How can one know whether one has bad breath at any given time point in time?

Future research on bad breath should cross over with the field of medical breath testing, which has thus far developed in an independent fashion. A recent review on the subject of medical breath tests completely ignored the oral cavity (Philips, 1992). Although medical breath testing often involves detection of non-odorous gases (*e.g.*, carbon monoxide, hydrogen), the sampling and instrumental techniques employed are often analogous (Preti *et al.*, this volume).

Finally, it should be kept in mind that bad breath shares kinship with other types of body odors. Body odors are usually of microbial origin and may be indicative of infection. They cause discomfort to many people and support vast cosmetic industries. As with bad breath, body odors may be imagined. Interestingly, certain body-associated odors may be more similar in chemical makeup to oral malodor than is currently recognized (Moore *et al.*, 1987).

Acknowledgments

I thank Sarit Goldberg, Orit Ilan, Hani Tal and Ilana Gelernter for their help in the dental student experiment and in reviewing the manuscript.

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2

The Biological Basis of Oral Malodor Formation

I. Kleinberg and M. Codipilly

The Kleinberg salivary sediment system has been used in our laboratory to study the biological basis of oral malodor formation and the primary mechanisms involved. The central role of the oral bacteria and amino acids, and the stimulatory role of saliva in malodor production were confirmed. A subsequent survey identified the bacteria and the amino acids mainly responsible. Involvement of the Gram-negative bacteria and lack of involvement of the Gram-positive bacteria were confirmed, as was the primary involvement of the sulfur-containing amino acids, cysteine, cystine and methionine. However, the survey also showed that among the more numerous Gram-negatives found in the mouth, *Neisseria* were not involved, whereas species of *Fusobacteria*, the black pigmented anaerobes, *Haemophilus* and *Veillonella* were. Further demonstrated was that amino acids other than the sulfur-containers were also major participants in malodor production. These included tryptophan and ornithine with a lesser contribution from arginine, a possible precursor of ornithine in some bacteria. Examination of the need for epithelial squames in oral malodor formation showed them not to be essential. Therefore, saliva may be a better source of amino acids since its

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peptides and proteins are more easily hydrolyzed. The physicochemical parameters of pH, $P0_2$, and E_h are determined by the metabolism of the oral bacteria and each can have major effects on oral malodor generation. Malodor occurs at neutral and alkaline pH and is inhibited by acidity. Oxygen depleted conditions are conducive to malodor formation if this is accompanied by lowering of the E_h. Fermentation of sugars, such as glucose, inhibits malodor generation which probably occurs by a combination of lowering the pH and raising the E_h. In contrast, putrefaction of the amino acids, peptides and proteins in salivary supernatant enhances malodor, most probably by raising the pH and lowering the E_h. Examination of the rates of loss from an epithelial surface (the back of the hand) of the main odoriferous volatiles believed to be involved in oral malodor showed hydrogen sulfide and methyl mercaptan to be lost extremely rapidly. In contrast, loss of indole/skatole, putrescine and cadaverine, and the several organic acids tested was slower. This was indicative of their ability to adhere and hence sustain malodor for prolonged periods of time. It is evident that amino acid catabolism by the oral bacteria is complex but its understanding is central to the elucidation of oral putrefaction mechanisms and the basis of oral malodor formation.

Introduction

It is rather interesting that our understanding of the fundamentals of oral malodor (Miller, 1890; Sulser *et al.*, 1939; Berg and Fosdick, 1946; Berg *et al.*, 1947; Tonzetich *et al.*, 1967; Tonzetich, 1971; 1977; McNamara *et al.*, 1972), and the origin of our present day theories of dental caries etiology (Miller, 1890) have both come from studying incubated whole saliva. This is a readily available, easy to manipulate system obtained by chewing an inert material, such as paraffin wax. Whole saliva collected in this fashion contains a sampling of the mixed microbial population dislodged from the oral hard and soft tissue surfaces of the mouth and a mix of the salivas that are stimulated to flow into the oral cavity. Upon centrifugation, a sediment of bacterial and epithelial cellular elements is pelleted (generally referred to as salivary sediment; Kleinberg, 1967a); the bacteria therein are both free and attached to epithelial squames arising from the continually growing and differentiating epithelium covering the oral soft tissue surfaces. The supernatant is a complex fluid consisting mainly of saliva from the various oral glands with some fluid seeping from the gingival crevice, especially when there is significant inflammation (Cimasoni, 1983). An extensive series of studies have shown, despite early reservations (Gibbons *et al.*, 1964), that salivary sediment is metabolically and microbiologically remarkably similar to pooled dental plaque (Denepitiya and Kleinberg, 1982; Singer and Kleinberg, 1983 a,b).

There is a simple classical experiment with incubated whole saliva that has been central to the development of our early concepts of dental caries and oral malodor etiology. It can be performed as follows. After collecting whole saliva, two samples are taken and glucose or similar fermentable sugar is added to one and no glucose is added to the other. Then both are incubated for about 4 to 24 hours at 37°C. In the sample to which glucose has been added, the incubation becomes acidic and, if teeth are added, as was done by Miller (1890) over one hundred years ago, demineralization will occur. This became the basis of the Miller acid decalcification theory for dental caries, namely that sugars are converted to acids by the mouth bacteria and the acids then decalcify the teeth, thereby beginning the caries process. Of importance to this presentation is the observation that no malodor occurs, which Miller (1890) and a later study by McNamara *et al.* (1972) showed to be largely the result of inhibition by the acidity produced during sugar fermentation, a process that evidently also counters base formation (Rae *et al.*, 1955). In contrast, in the incubation mixture to which no glucose is added considerable odor is invariably observed (Miller, 1890). Upon investigation of the basis of this malodor production, Fosdick and his colleagues in a series of studies (Sulser *et al.*, 1939; Berg and Fosdick, 1946; Berg *et al.*, 1947) established malodor to be the result of bacterial putrefaction, a process wherein oral bacteria act on salivary proteins and peptides to produce products that include compounds that are both volatile and odoriferous. Later studies by Tonzetich and his associates (see reviews by Tonzetich, 1977 and Kleinberg and Westbay, 1990) confirmed these findings and, among other things, showed the air space over incubated whole saliva and human breath air to be similar (Richter and Tonzetich, 1964; Tonzetich, 1971). Much of the basic information on oral malodor developed with incubated whole saliva is still valid today and more importantly, bacterial putrefaction has been confirmed as the central process in oral malodor formation, just as fermentation established in incubated whole saliva was confirmed as the central oral bacterial process in dental caries.

Development of the Kleinberg Salivary Sediment System Model from Whole Saliva and Its Use for Oral Malodor Study

As a first step to building an *in vitro* mixed microbial model to study the metabolic basis of the pH changes seen in plaque *in vivo,* Kleinberg (1967a) recognized that whole saliva could provide a simple, fresh and easily obtainable source of mixed bacteria suitable for this purpose but unlike whole saliva, the bacterial concentration needed to be raised substantially and controlled (Stephan and Hemmens, 1947; Stralfors, 1950 for pure cultures). In a study that examined a wide range of salivary sediment and glucose concentrations, sediment-substrate levels were identified that enabled simulation of the kinds of pH curves seen *in situ* (Kleinberg, 1961; 1967b; Kleinberg *et al.,* 1981; 1982) and the validity of use of salivary sediment for the model was later confirmed in a variety of metabolism studies carried out with salivary sediment, dental plaque and pure culture mixtures *in vitro* (*e.g.* Singer *et al.,* 1983; Kleinberg *et al.,* 1977 and Kleinberg *et al.,* 1982). In these studies, as was shown earlier with pure cultures (Stephan and Hemmens, 1947; Stralfors, 1950), high cell concentration and appropriate use of limiting and non-limiting substrate levels were crucial to simulating *in vivo* metabolic conditions. The metabolism of the oral mixed microbiota as an entity was characterized (Sandham and Kleinberg, 1969;1970a,b; Kleinberg *et al.,* 1973; Ryan and Kleinberg, 1994a,b; Singer and Kleinberg, 1985a,b) and the remarkable similarity of the metabolism of salivary sediment and pooled dental plaque from a given person's mouth was demonstrated (Kleinberg *et al.,* 1977; Singer *et al.,* 1983; Salako and Kleinberg, 1992; Wijeyeweera and Kleinberg, 1989a; Ryan and Kleinberg, 1995a,b). Also identified were the more numerous members of the oral microbiota which carry out the main metabolic processes that characterize sediment and plaque mixed microbial metabolism (Salako and Kleinberg, 1992; Ryan and Kleinberg, 1995a,b; Traudt and Kleinberg, 1988). In addition, it was demonstrated that changing the microbial composition of sediment or plaque composition by selective pure culture addition caused corresponding changes in the metabolism of the whole sediment or plaque entity (Wijeyeweera and Kleinberg, 1989a,b; Ryan and Kleinberg, 1995a). This enabled different kinds of microbiota such as cariogenic and non-cariogenic, as well as odorigenic and non-odorigenic (see Figures 6 and 7 below) to be simulated.

The present study used the sediment model to re-examine and extend studies on the biological basis and mechanisms involved in oral malodor generation. A first step was to repeat the whole saliva classical experiment with the Kleinberg salivary sediment system (Kleinberg, 1967a) where the cell concentration is much greater than that found in whole saliva and is designed to behave metabolically like dental plaque (Singer *et al.*, 1983). First, salivary sediment was prepared from wax-stimulated whole saliva by centrifugation at 1740 g and the sediment and supernatant were used to prepare two incubation mixtures (750 µl), one containing sediment at 16.7% (v/v), supernatant at 33.3% (v/v) and glucose at 28 mM and the other contained the same ingredients but with the glucose omitted. Incubation was carried out for 4 h at 37°C and the pH and malodor were determined at appropriate intervals. Malodor was determined organoleptically on a scale of 0 to 4 (0 for no odor; 4 for most odor), which was carried out by inserting a solid glass rod into the incubation mixture, slowly rotating three times and then sniffing the rod after its removal. This proved to be a much more reproducible method than organoleptic headspace malodor assessment.

The results are shown in Figure 1 and clearly demonstrate that the pH rapidly becomes acidic and as before, that there is no malodor with glucose addition, whereas without the glucose, the pH shows a progressive rise and there is an abundance of malodor. Further experiments showed that the salivary supernatant in the system produces no odor when incubated alone, whereas the sediment wherein the bacteria are located does produce malodor and does so to a greater extent when salivary supernatant is added (see next paragraph below). Subsequent studies showed supernatant enhancement of odor formation to be mostly due to the amino acids that salivary supernatant provides when its salivary peptides and proteins are hydrolyzed (Kleinberg *et al.*, 1979; Kleinberg and Westbay, 1992).

Major constituents of the malodor end-products of oral bacterial putrefaction include several volatile sulfur compounds (VSC; Tonzetich and Carpenter, 1971). The main ones are hydrogen sulfide and methyl mercaptan and these can be detected in a subject's breath by gas chromatography (Tonzetich and Carpenter, 1971) or in real time with a recently developed hydrogen sulfide portable analyzer called the Halimeter (Interscan, Corporation, Chatsworth, CA; Rosenberg *et al.*, 1991a,b). We modified this device in our laboratory to enable VSC detection of the hea dspace in incubating bacterial mixtures and have used it to assess VSC involvement and

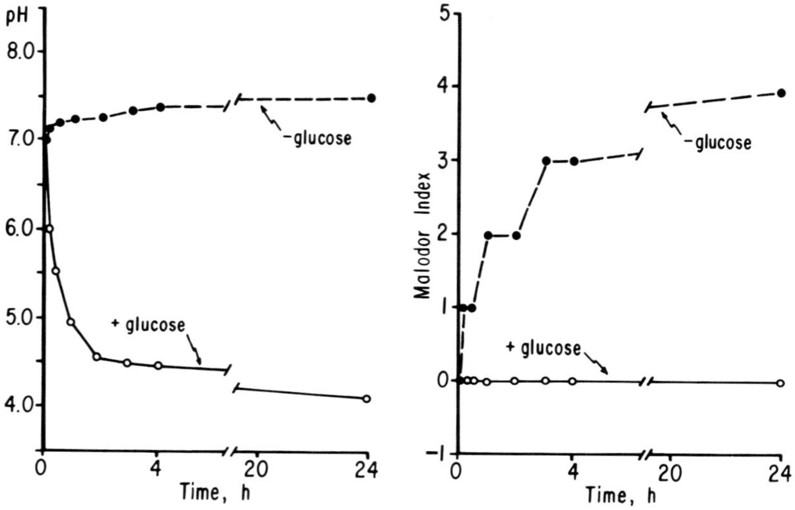


Figure 1. Effect of glucose on the pH and on oral malodor formation in incubated salivary sediment mixtures. Mixtures contained sediment at 16.7% (v/v), salivary supernatant at 33.3% (v/v) and glucose at 0 or 28.0 mM.

contribution to oral malodor in the following and other such types of experiments. Incubation mixtures were prepared consisting of sediment alone, sediment with glucose, and sediment with supernatant. The mixtures were incubated for 4 hours and odor was assessed both with the Halimeter and organoleptically. At the same time, the ability to release volatiles from solution after brief stirring with a solid glass rod was similarly assessed. The results are shown in Table 1.

As described above, glucose totally suppressed odor formation by the bacteria in the sediment by either Halimeter or organoleptic assessment even after stirring, a procedure that favors release of odoriferous compounds when present. In contrast, in the mixtures with sediment or sediment plus supernatant, odor production was observed both organoleptically and with the Halimeter. The Halimeter measurements in the sediment plus supernatant mixture showed a headspace reading of 13 ppb at zero time without stirring which increased to about 100 ppb at the end of the incubation. In contrast,

Table 1
Effect of Stirring on Odor Measurement by (i) Organoleptic and (ii) Halimeter Methods in Salivary Sediment Mixtures.

	Sediment alone				Sediment + glucose				Sediment + salivary supernatant			
Time	Smell		Halimeter[a]		Smell		Halimeter		Smell		Halimeter	
(hrs)	NS[b]	S[c]	NS	S	NS	S	NS	S	NS	S	NS	S
0	0	0	15	16	0	0	14	16	0	0	13	15
0.25	0	0	16	18	0	0	14	15	0	1	12	35
0.50	0	0	19	22	0	0	15	20	0	1	16	64
1	0	0	15	28	0	0	14	18	0	2	15	98
2	0	0	15	19	0	0	13	15	0	2	18	363
3	0	0	10	24	0	0	8	16	1	2	22	390
4	0	0	12	30	0	0	9	17	1	2	31	478
24	0	0	14	54	0	0	12	22	2	4	100	1134

[a] ppb sulfide [b] no stirring [c] with stirring

with stirring, it went from 15 ppb to about 1134 ppb. Clearly, the odoriferous volatiles are present in an oversaturated state since simple stirring leads to substantial release from solution. The Halimeter readily detects hydrogen sulfide and since this volatile is a major component of malodor and bad breath, its detection in the headspace of incubating saliva and in such breath (Richter and Tonzetich, 1964) is to be expected.

Epithelial Cells as a Source of Substrate for Malodor Formation

A question that arises is whether or not epithelial cells are essential for odor production. We tested this possibility by separating most of the bacteria in whole saliva that are attached to epithelial cells from those that are free. This was performed by simple differential centrifugation in the following type of experiment.

Unfractionated salivary sediment was prepared from whole saliva (15 ml) as described above. A second portion of the whole saliva (35 ml) was then separated into unattached and epithelial attached bacterial fractions as follows. The saliva was centrifuged at 10,000 g for 30 minutes and the resultant pellet was suspended in deionized water at a final volume of 15 ml. This suspension was vortexed to evenly disperse the pellet and then centrifuged at 1740 g for 1 minute. The supernatant contained free bacteria; the sediment contained epithelial cells with attached bacteria. The supernatant was decanted and then centrifuged at 10,000 g for 30 minutes to produce a bacterial pellet virtually free of epithelial cells. This was confirmed by inspection under the light microscope.

The three cell preparations (unfractionated salivary sediment, unattached bacteria, and bacteria attached to epithelial cells) were each washed with distilled water and then used to prepare incubation mixtures (900 μl) containing each cell preparation at 11.1% (v/v), salivary supernatant at 33.3% (v/v) and 60 mM potassium phosphate buffer at pH 7.0. Incubation of the mixtures containing the three cell preparations at 37°C for 24 h demonstrated that the oral bacteria free of epithelial cells give as much odor as with epithelial cells present (Figure 2). By itself, the epithelial cell fraction with attached bacteria actually produced less odor, but this may simply reflect the lower number of bacteria *per se* because of the volume occupied by the metabolically inert epithelial squames. Be this as it may, this type of experiment clearly showed that bacteria are the prime elements needed to give oral malodor (see Miller, 1890) and that epithelial cells are not essential. This does not mean that epithelial cells make no contribution. They could be a source of amino acids as are the various peptides and proteins in saliva (Kleinberg *et al.*, 1979).

Identification of the Oral Bacteria and Amino Acid Substrates Involved in Oral Malodor Generation

As has been carried out in our laboratory for other sediment metabolic parameters, the specific bacteria that participate in malodor production along with the key amino acid substrates involved were next determined. The bacteria tested were those usually present in largest numbers in supragingival plaque and the exogenous substrates tested for odorigenicity included most of the common amino acids.

This survey was carried out as follows. Pure cultures of each of the 25 microorganisms listed in Figure 3 were grown at 37°C in appropriate media and under appropriate growth conditions (Salako and Kleinberg, 1992) to their middle to late exponential phases of growth. Organisms were harvested

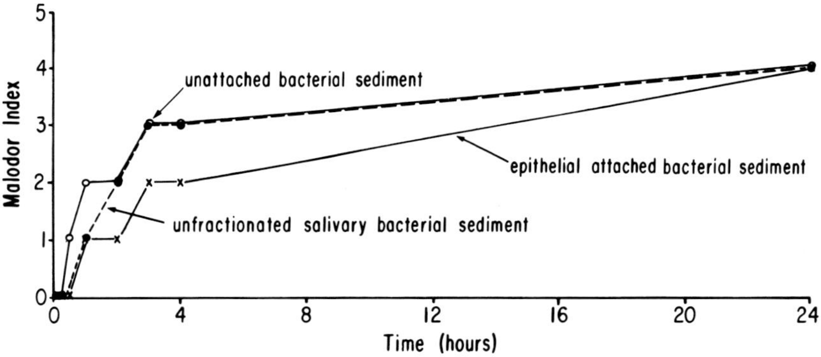
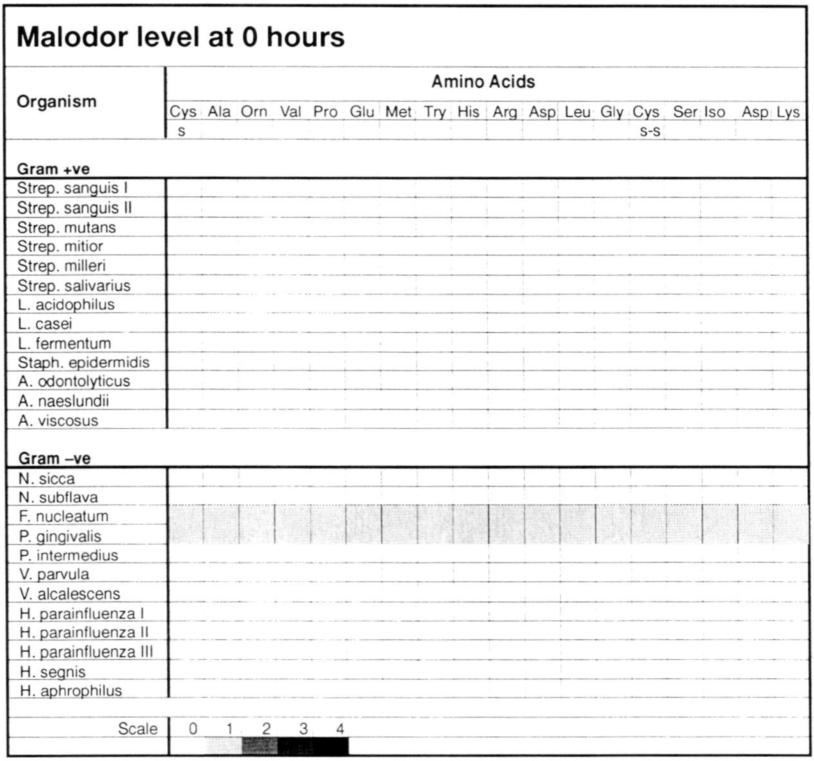


Figure 2. Malodor production by the unattached and epithelial attached bacteria in salivary sediment.

by centrifugation at 10,000 g for 20 minutes at 4°C. Each organism was washed by adding sterile deionized water and dispersed by vortexing. The resulting suspension was centrifuged at 1740 g for 15 minutes at 4°C and the supernatant was discarded. This procedure was repeated twice on the resultant bacterial pellet before the bacteria were suspended at a final concentration of 25% (v/v).

Malodor formation with each microorganism was determined in mixtures (750 µl) containing each bacterial type at a suspension concentration of 8.3% (v/v) and each of the amino acids listed in Figures 3 to 5 at a concentration of 3.3 mM. As above, the pH was maintained at 7.0 during incubation for 24 hours at 37°C using a 60 mM potassium phosphate buffer. As before, malodor was assessed organoleptically on a scale of 0 to 4. This was done at the beginning and after 15 and 30 minutes, and at 1, 2, 3, 4 and 24 hours of incubation. When malodor occurred, its production progressively increased throughout the incubation. For brevity, only the 0, 4 and 24 hour results are shown in Figures 3 to 5. Quite significant was the demonstration that the Gram-positive bacteria in general produced little or no malodor even after 24 hours of incubation. Among the Gram-negatives, only the *Neisseria* gave similar results. The other Gram-negative microorganisms tested were all clearly odorigenic. Except for the black pigmented anaerobe, *Porphyromonas*



Malodor level at 0 hours

Organism	Amino Acids																	
	Cys S	Ala	Orn	Val	Pro	Glu	Met	Try	His	Arg	Asp	Leu	Gly	Cys S-S	Ser	Iso	Asp	Lys
Gram +ve																		
Strep. sanguis I																		
Strep. sanguis II																		
Strep. mutans																		
Strep. mitior																		
Strep. milleri																		
Strep. salivarius																		
L. acidophilus																		
L. casei																		
L. fermentum																		
Staph. epidermidis																		
A. odontolyticus																		
A. naeslundii																		
A. viscosus																		
Gram –ve																		
N. sicca																		
N. subflava																		
F. nucleatum	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░
P. gingivalis	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░	░
P. intermedius																		
V. parvula																		
V. alcalescens																		
H. parainfluenza I																		
H. parainfluenza II																		
H. parainfluenza III																		
H. segnis																		
H. aphrophilus																		
Scale	0	1 ░	2 ▒	3 ▓	4 █													

Figure 3. Malodor determined organoleptically at 0 time in incubation mixtures containing various pure cultures of oral bacteria and amino acids.

gingivalis (formerly *Bacteroides gingivalis*), these did so mainly with the following six amino acids: cystine, cysteine, methionine, tryptophan, ornithine and arginine. *P. gingivalis* was particularly effective, being able to produce malodor with amino acids other than the above six but unlike *Prevotella intermedia* (formerly *Bacteroides intermedius*), this micro-organism is normally present as a minor member of supragingival non-periodontitis plaque. The above black pigmented anaerobes and *Fusobacteria* have generally been known to be active odor producers. However, the results in this study indicate that *Haemophilus* and *Veillonella* species may also be involved in odor formation, for example, being able to produce odor from

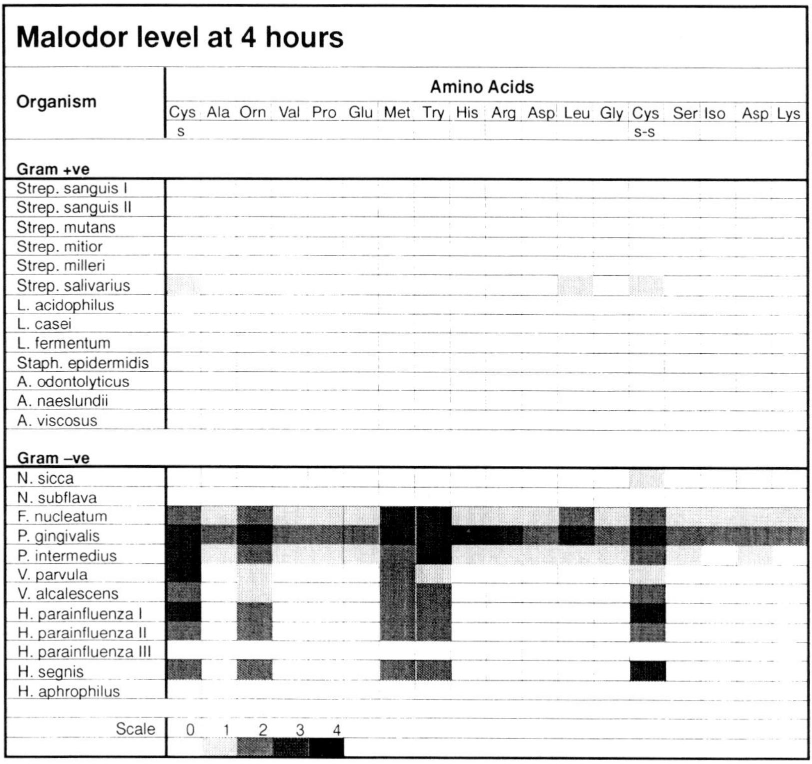


Figure 4. Malodor determined organoleptically at 4 hours in incubation mixtures containing various pure cultures of oral bacteria and amino acids.

ornithine. Most importantly, the above Gram-negative bacteria are major constituents of supragingival plaque and the plaque that covers the tongue and other mucosal surfaces.

Basically, these results confirm the earlier observations of McNamara *et al.* (1972) and Tonzetich (1971; 1977) in that Gram-positive bacteria contribute very little to oral malodor production, whereas the bacteria mainly responsible are Gram-negative.

Examination of the role of the various amino acids as substrates in odor formation confirmed the earlier Tonzetich and Carpenter (1971) results showing that the sulfur-containing group of amino acids (cysteine, cystine

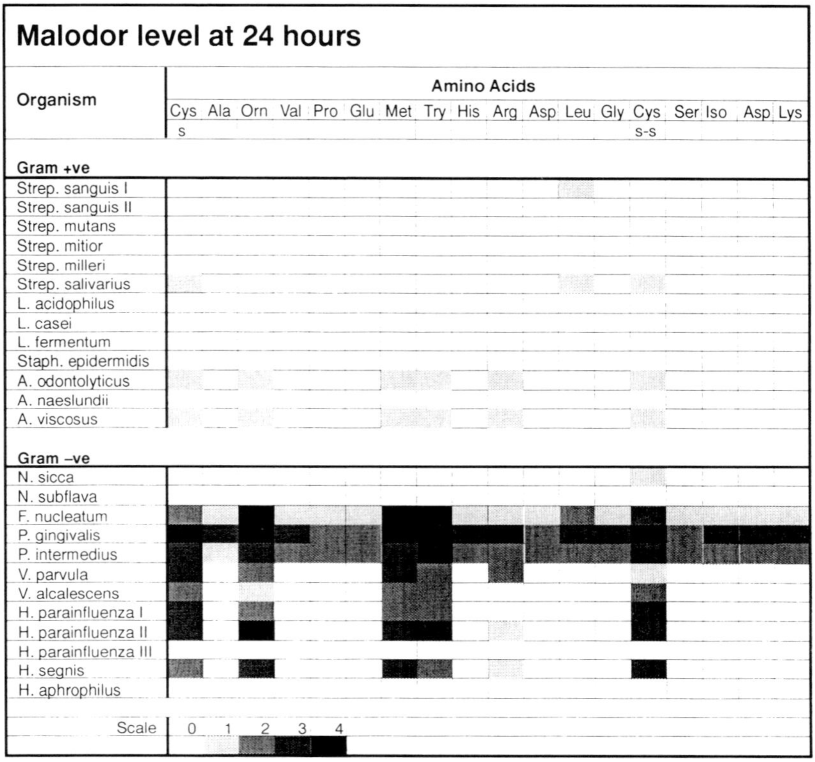


Figure 5. Malodor determined organoleptically at 24 hours in incubation mixtures containing various pure cultures of oral bacteria and amino acids.

and methionine) are major odor contributors. The main odoriferous products produced from these substrates are hydrogen sulfide and methyl mercaptan. However, tryptophan was also a major contributor to odor formation in our study as was ornithine. Minor contributions from histidine, arginine and others were evident. Arginine can result in ornithine formation by the arginine deaminase pathway (Kanapka and Kleinberg, 1983) but for some reason gave much less odor than ornithine. Thus, it would appear that the sulfur-containing amino acids, plus tryptophan and ornithine are the dominant (but not the only) substrates involved in oral malodor generation.

Alteration of the Microbial Composition and Odorigenic Capability of the Mixed Microbiota of Salivary Sediment by Selected Pure Culture Substitutions

Besides identification of the microorganisms and amino acids significantly involved in oral malodor formation, the basic information provided by the bacterial-substrate grid for malodor enables selection of the most appropriate bacteria for addition to the Kleinberg sediment system to alter the sediment biota to a composition of greater or lesser odorigenic capability. In this way, microbiota of individuals or in different intra-oral sites which have different odorigenic capabilities may be simulated. This is illustrated in the following pair of experiments in which five (plus a control) incubation mixtures were prepared for each with the following compositions. Three tubes contained either salivary sediment, a pure culture of the nonodorigenic microorganism *Streptococcus sanguis II* (*Streptococcus oralis* by the newer nomenclature; Marsh and Martin, 1991) and either the odorigenic bacterium, *F. nucleatum* or *P. intermedia*. The other two tubes had a mixture of salivary sediment and *S. sanguis II* in one, and a mixture of sediment and either *F. nucleatum* or *P. intermedia* in the other. The final concentrations of salivary sediment alone and pure culture alone were 16.7% and 8.3% (v/v), respectively (see Singer *et al.*, 1983 for the basis of use of such a concentration difference). The sediment/bacterial combinations consisted of 8.3% (v/v) salivary sediment,4.2% (v/v) pure culture, and 4.2% (v/v) sediment inactivated by heating to ensure a total cell suspension concentration of 16.7% (v/v) and a similar buffering capacity to that of unaltered sediment (Singer *et al.*, 1983). Each of these incubation mixtures also contained salivary supernatant at a final concentration of 33.3% (v/v) and phosphate buffer at 60 mM and pH 7.0 to keep the pH constant. A sixth mixture containing only salivary sediment at 16.7% (v/v) and buffer was also included for comparison.

All tubes were incubated at 37°C and malodor determinations were made at 0, 15, and 30 minutes and at 1, 2, 3, 4, 8 and 24 hours. The results are shown in Figure 6.

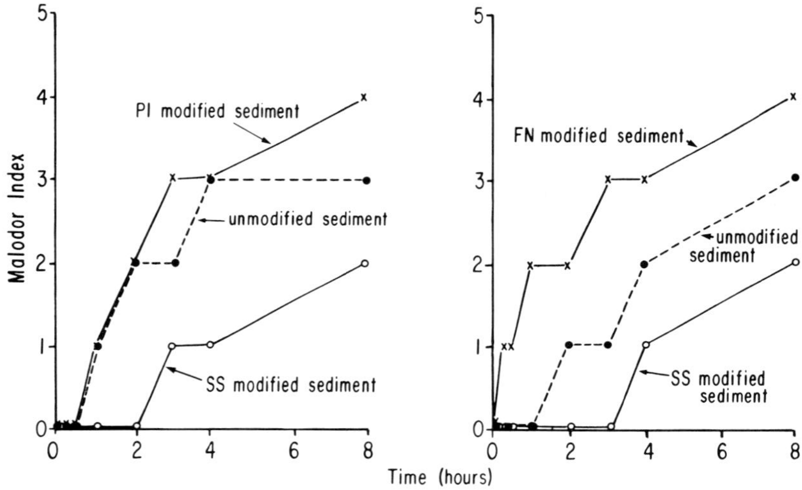


Figure 6. Effect of alteration of the salivary sediment microbiota composition on malodor production capability. Note that 1:1 substitution of sediment with *Streptococcus sanguis II* (SS) decreased whereas similar substitution with *Fusobacterium nucleatum* (FN) and *Prevotella intermedia* (PI) increased sediment odor producing capability.

Addition of *S. sanguis II* reduced the odorigenicity of the mixed bacteria in salivary sediment significantly, especially at the beginning of the incubation. In contrast, addition of *F. nucleatum* or *P. intermedia* increased somewhat or replaced the malodor generation capability lost by reducing the sediment concentration when these bacterial additions were made. Experiments such as these illustrate what is generally believed, *i.e.*, that bacteria differ in their contributions to malodor formation. It also makes it possible to assess which members of the oral microbiota are odorigenic and which are nonodorigenic in a mixed microbial situation, such as that present in the mouth. This whole area needs further exploration and our sediment model system and this approach should help make this easier.

Effect of pH and the Role of the Acid-Base Metabolism of the Oral Bacteria on Malodor Generation

Earlier studies have identified that the pH is a major regulatory factor in malodor formation (McNamara *et al.,* 1972). It is clearly established that acidity inhibits malodor production, whereas neutrality and alkalinity favor it. The acid-base metabolism of the mixed bacteria in the oral cavity largely determines the pH and this is mainly controlled by the plaque microbial composition and two categories of substrates. One is fermentable carbohydrate. It can cause the pH to rapidly shift in an acidic direction (Figure 1); the major sources of carbohydrate substrate for the mouth are the common sugars and starches in the diet, and the sugar amines and other moieties associated with salivary glycoproteins and mucins (Caldwell and Pigman, 1966). The second category of substrates that has major effects on the pH are nitrogenous compounds. These include urea, and free amino acids, as well as amino acids derived from peptides or proteins (Kleinberg *et al.,* 1979). Of these, urea is unique in that it can rapidly raise the pH (Kleinberg, 1967a,b). It is available on a continuous basis from saliva and from gingival crevicular fluid if there is any gingival inflammation (Golub *et al.,* 1971). Arginine also favors alkalinity especially when the pH is acidic but its rate of base formation is slower (Kanapka and Kleinberg, 1983). Several other amino acids can contribute to base formation when the pH is acidic but arginine is the most powerful.

Amino acids are also involved in counteracting the high pH favored by urea and in bringing the pH back to neutrality (Kleinberg, 1970). Serine, glutamine and asparagine are best able to do so, but the sugar moieties of the salivary glycoproteins may be at least as important as amino acids for this purpose (Chatterjee and Kleinberg, 1995).

As for other sediment or plaque metabolic parameters, the number and types of bacteria present in oral microbial communities have major effects on the pH. Examination of the fermentation capabilities of the bacteria listed in Figures 3 to 5 with a variety of carbohydrate substrates disclosed that the largest decreases in pH occur with the Gram-positive members of the oral microbiota (Salako and Kleinberg, 1992; Ryan and Kleinberg, 1995a,b). Some of these are also arginolytic (Wijeyeweera and Kleinberg, 1989a) and capable of bringing an acidic pH back to neutrality. This has a major attenuating effect on the pH (Kleinberg *et al.,* 1982). An overload of the highly

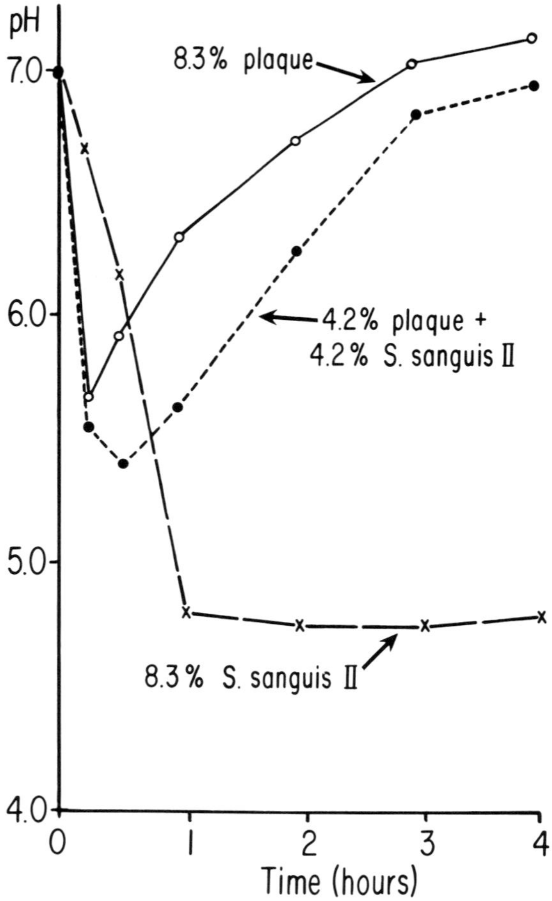


Figure 7. Effect of alteration of the dental plaque microbiota composition on its acid producing capabilities by 1:1 substitution of plaque with *Streptococcus sanguis* II (re-drawn from Kleinberg *et al.*, 1982).

fermentative non-arginolytic Gram-positive microorganisms, such as *S. sanguis II* (Figure 7), while possibly significant in cariogenic microbiota development, can be expected to reduce malodor generation because such microbiota compositions favor a more acidic pH level and acidity is inhibitory of the formation of malodor (Miller, 1890; McNamara *et al.*, 1972).

Several of the oral bacteria have been identified as having significant ureolytic capability, a characteristic favoring an alkaline pH. These include *Haemophilus parainfluenzae, Actinomyces viscosus, Actinomyces naeslundii* and *Staphylococcus epidermidis* (Salako and Kleinberg, 1989). A strain of *Streptococcus salivarius* may have ureolytic activity (Sissons *et al.*, 1988) but its numbers in the mouth appear to be small and hence, its contribution is still uncertain. Nonetheless, microbiota with elevated numbers of ureolytic bacteria can be expected to raise the ureolytic capability of the mixed bacterial plaque entity and favor increased malodor (as well as calculus; Frostell, 1960), other factors being equal.

Role of Oxygen Depletion and the Oxidation-Reduction Potential in Malodor Generation

Löe *et al.* (1965) have shown in humans that gingivitis develops when oral hygiene is stopped and dental plaque is allowed to spontaneously accumulate on the oral hard and soft tissue surfaces. In dogs, Lindhe *et al.* (1975) showed the same result but, in addition, demonstrated that if the accumulation is prolonged, periodontitis will eventually develop. Plaque oxygen depletion and decrease in the E_h, two factors involved in oral malodor generation, occur during such plaque accumulation. Using a specially constructed micro-oxygen electrode during a 4-day plaque accumulation, Globerman and Kleinberg (1979) showed a decrease in the $P0_2$ to a level at or near 0 mm of Hg in plaque on both the teeth and the soft oral tissues. Kenney and Ash (1969), using a platinum electrode to measure plaque E_h levels during 4-and 7-day accumulation periods, observed a fall in the E_h from +244 MV to -112 MV after 4 days in the one case and to -141 MV after 7 days in the other (Table 2). These changes corresponded to the emergence of the anaerobic Gram-negatives important for malodor generation and gingivitis periodontitis development (Löe *et al.*, 1965; Lindhe *et al.*, 1975). In the same study, Kenney and Ash (1969) also demonstrated that lower E_h values are observed when periodontitis is evident (Table 3).

Katayama *et al.* (1975) used redox dyes to assess the E_h of dental plaque *in vivo*. They used a mixture of methylene blue, which has a standard oxidation-reduction potential (E_o) of around +11 MV and triphenyltetrazolium chloride (TTC), which has an E_o of about -80 MV. When subjects rinsed their mouths

Table 2
Oxidation Reduction Potential of Developing Dento-Gingival Plaque in Each of Two Subjects.

Subject 1			**Subject 2**		
Days after insertion of electrodes	Oxidation reduction potential (millivolts)	Bacterial smear	Days after insertion of electrodes	Oxidation reduction potential (millivolts)	Bacterial smear
0	+244		0	+244	
1	+ 63	Gram-positive cocci Gram-positive cocci	2	- 30	Gram-positive cocci Gram-positive rods in short chains
3	- 31	Gram-positive cocci Gram-negative cocci Gram-positive rods Gram-negative slender rods	3	- 67	Gram-positive cocci Gram-negative cocci Gram-positive rods
4	-112	Gram-positive cocci Gram-negative cocci Gram-positive rods in chains Gram-negative slender rods	7	- 141	Gram-positive cocci Gram-negative cocci Gram -positive rods in chains Gram-negative short rods A few unbranched Gram-negative filaments

From Kenny and Ash (1969).

Table 3
Oxidation Reduction in Millivolts of Gingival Sulci and Periodontal Pockets of Subjects With and Without Periodontal Disease.

	Subjects with periodontal disease		**Control group**
	Periodontal pockets	Gingival sulci	Gingival sulci
	+ 14	+113	+ 33
	- 94	+ 53	+ 64
	- 61	+ 10	+ 51
	- 157	+ 15	+ 73
	+ 12	+102	+103
	- 28	+103	+ 63
	- 6	+ 47	+ 93
	- 32	+ 67	+ 86
	- 57	+103	+ 88
	- 67	+113	+ 90
Mean	- 47.6 (SE ± 15.6)	+ 72.6 (SE ± 12.0)	+ 74.4 (SE ± 6.5)

From Kenny and Ash (1969).

with this dye mixture, 3-day plaque showed methylene blue in its blue,oxidized form at zero time but after about 10 minutes, a small amount of red was evident, which is indicative of TTC reduction. In contrast, in thicker 7-day plaque, the methylene blue staining was instantly seen as before, but very quickly thereafter the red, reduced form of TTC was observed. This means that the E_h reached at least -80 MV, a reduced state that is favorable for the growth and metabolism of the anaerobic Gram-negative microorganisms involved in periodontal disease and oral malodor production.

The interaction between oxygen depletion and the E_h is complex. In the salivary sediment system, oxygen depletion occurs when either glucose or salivary supernatant are the sources of oxidizable substrate (Korayem and Kleinberg, 1990). However, their effects on the E_h are opposite. This can be seen in Figure 8 in which an incubation experiment such as that in Table 2 was carried out. Four incubation mixtures were prepared containing salivary sediment and 60 mM phosphate buffer (at pH 7.0) in each. To one, glucose at 28.0 mM was added; to the second, the addition was salivary supernatant at 33.3% (v/v); to the third, the addition was both glucose and supernatant; and to the fourth which served as a control, neither glucose nor supernatant was provided. The E_h was measured during a 24 h incubation at 37°C using platinum and calomel electrodes.

With glucose, the E_h rose to a level of about +470 MV (relative to the hydrogen electrode). This corresponds to an E_h commonly seen when streptococci are similarly incubated (Hewitt, 1950) and has been attributed to the formation of hydrogen peroxide when glucose is utilized by such bacteria in the presence of oxygen (Hewitt, 1950; Ryan and Kleinberg, 1995b). In contrast, with salivary supernatant, the E_h fell to about -80 MV which corresponds to the levels seen by Katayama *et al.* (1975) *in vivo*. In the mouth, the bacteria involved in oxygen depletion with sugar substrates are mostly the Gram-positives, especially the streptococci and actinomyces. With amino acids as the substrates, the oral Gram-negatives, especially *Neisseria* and *Haemophilus*, are mainly responsible for oxygen depletion (Traudt and Kleinberg, 1988). Collectively, these numerically prominent bacteria comprise the bulk of the early plaque colonizers and hence should be its main plaque oxygen depleters. However, the Gram-negatives in this group along with other Gram-negatives are probably also significant contributors to the E_h reduction that occurs as plaque accumulates on oral surfaces (Table 2).

Oral Malodor Generation in vivo

Malodor generation necessarily depends upon the ability of odoriferous end products to be volatilized from the oral soft and hard tissue surfaces. For this to occur, the thickness of the film of saliva that normally covers these surfaces may be a modifying factor. The saliva film that remains on the surfaces of the oral tissues immediately after swallowing is called the residual saliva (Dawes, 1987). Before exhaling through the mouth as in talking, a

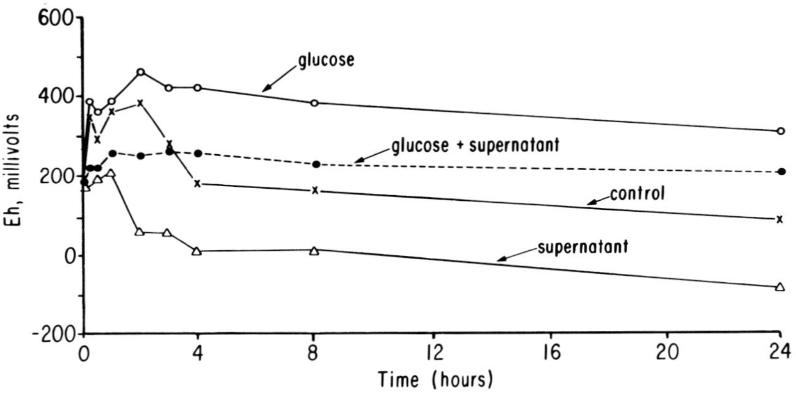


Figure 8. Effect of glucose, salivary supernatant and both glucose and supernatant on the E_h of incubated salivary sediment mixtures. Note that with glucose and salivary supernatant present, the E_h is maintained over a relatively narrow range.

person usually swallows and expired air then passes over the oral tissues if the mouth is open. This can cause the tissues, especially the hard palate and the lips, to lose enough residual saliva so that the sensation of dryness is perceived (Wolff and Kleinberg, 1994). At this time, it is possible that odoriferous volatiles are lost more easily from oral surfaces into the breath.

One can measure the thickness of the residual saliva on the mucosal surfaces with special paper strips called Sialopaper™ (Pro-Flow) and an instrument called the Periotron® (Pro-Flow; Amityville, NY). Wetness of the surfaces of the palate, tongue and other oral sites has been measured in our laboratory by this procedure (DiSabato-Mordarski and Kleinberg, 1989; Wolff and Kleinberg, 1994). The thinnest film of residual saliva was found to be that on the oral mucosa of the hard palate. As indicated above, this is where it should be easiest to sense the condition of dryness and to lose odoriferous volatiles into the breath. One can simulate to a certain degree what happens for different volatiles by using the "wrist lick test" (Rosenberg and Leib, this volume). In this test, the moisture on the hand evaporates and malodorous volatiles escape into the air where they are easily detected organoleptically.

This suggested a simple but relatively quantitative method to assess the relative ease of volatilization of the main odoriferous volatiles believed to be involved in oral malodor.

Accordingly, the following set of experiments was carried out. A single organoleptic examiner (MC) was used. Test compounds included the organic acids (acetic, propionic, butyric, valeric), sulfur containing volatiles (H_2S and CH_3SH), aromatic volatiles (indole and skatole) and amines (putrescine and cadaverine) listed in Table 4. H_2S and CH_3SH solutions were prepared by bubbling each gas through 2 ml of distilled water for 2 minutes. The others were prepared as 12.5 mM solutions. Before each test, the examiner sniffed a range of concentrations of each odorigenic compound (comparison standards) to facilitate organoleptic scoring. A skin area on the back of the left hand was cleaned with soap and water and dried well with a paper towel. A cotton wool applicator was then saturated with one of the test solutions and applied for 10 seconds onto a 2.5 cm diameter circular area of the skin of the back of the hand. The hand was kept resting on the bench top and the skin area was sniffed at one minute intervals with the nose positioned 2.5 cm away from the hand surface. Odor was assessed on a scale of 0 to 4. At the same time intervals, loss of moisture from the application area was measured using Sialopaper™ strips and the Periotron®. The results are given in Table 4.

All compounds tested had characteristic odors so that progressive decrease in odor intensity with time (indicative of loss of evaporation) could be easily assessed; some showed more rapid odor loss than others. H_2S and CH_3SH were lost remarkably quickly, within the first two minutes. In fact, these compounds were lost as fast if not faster than water which occurred for all test solutions between 1.5 and 2.5 minutes. Loss of odor with the other test substances took longer (Table 4). Indole and skatole took the longest. These lasted much beyond the period of water loss, indicating that unlike H_2S and CH_3SH, these compounds must adhere to epithelial surfaces and thereby provide odor for extended periods of time. Loss of water appeared to only affect the organic acid loss in the first minute (as indicated by increase in odor upon evaporation) but otherwise there was no difficulty for the various odoriferous volatiles to be detected. Perhaps at lower volatile concentrations, water loss might be more significant than observed here.

Table 4
Odor Scores at Various Time Intervals after Application of Aqueous Solutions of Oral Odoriferous Volatiles to the Skin on the Back of the Hand.[a]

	0 min	1 min	3 min	10 min
hydrogen sulfide	3	1	0	0
methyl mercaptan	3	1	0	0
acetic acid	3	4	0	0
propionic acid	3	4	0	0
butyric acid	4	4	2	2
valeric acid	4	4	3	2
indole	4	4	4	2
skatole	4	4	4	2
putrescine	4	4	3	1
cadaverine	4	4	2	1

[a] Solutions were 12.5 mM except for hydrogen sulfide and methyl mercaptan which were aqueous solutions obtained by bubbling these gases through distilled water for 2 minutes at 20°C.
Water evaporation was complete in all cases after 1.5 to 2.5 minutes. More than 50% evaporated within the first minute.

Concluding Remarks

It is clear that degradation of certain amino acids by most of the numerically prominent Gram-negative oral bacteria is central to the production of oral malodor. Significant pH, PO_2 and E_h changes occur during oral bacterial metabolism of carbohydrate and nitrogenous substrates and these can be major regulatory factors in the generation of the odoriferous end-products involved. Ability of the odoriferous volatiles produced during putrefaction to be lost into the breath, especially from oral surfaces covered by little residual saliva, might be a factor but this needs to be more thoroughly investigated. *In vitro* models, such as the salivary sediment system prepared from whole saliva, have helped considerably in the development of an understanding of oral malodor mechanisms and in simulation of oral malodor processes. Such simulation should facilitate development of new and better treatments of the oral malodor condition and perhaps identify new means of prevention and treatment of periodontal disease.

Acknowledgments

The secretarial assistance of Ms Patricia Calia and the diagram preparations of Mr James Skillman are most gratefully acknowledged.

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3

Strategies to Identify the Main Microbial Contributors to Oral Malodor

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Oral malodors are a common complaint of adults who present in a dental office. These odors arise from microbial degradation of proteins (especially those that contain cysteine and methionine), peptides, and amino acids that are present in saliva, gingival crevicular fluid (GCF) or in foods that are retained on the teeth. The responsible organisms appear from *in vitro* studies to be proteolytic, anaerobic, Gram-negative species that colonize the subgingival plaque associated with periodontal disease. Two species which produce large amounts of CH_3SH from serum proteins *in vitro* are the BANA-positive species, *Porphyromonas gingivalis* and *Treponema denticola*. The BANA reactivity of tongue samples plus the measurement of VSC in mouth air provide a convenient and objective chairside means of quantifying the levels of malodor (Kozlovsky *et al.*, 1994).

The tongue microbiota appear to make the major contribution to oral malodors, but the presence of *P. gingivalis* and *T. denticola* in throat samples has not been documented. Treatments which successfully reduce oral malodors coincide with a reduction in the BANA-positive microbiota and in the relative

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and absolute numbers of anaerobes in throat samples. These findings suggest that treatments combining debridement of the tongue surface and use of antimicrobial mouth rinses can be effective in the short term for reducing oral malodors. Additional studies are needed to determine whether the effect is long-lasting and which modality of treatment, *i.e.*, debridement or mouthrinse, is to be preferred.

I Introduction

Oral malodor is a common complaint among adults, often being the reason why an individual seeks dental care. One type of malodor relates to habits, such as smoking or drinking alcoholic beverages, or to the recent ingestion of certain foods, such as onions or garlic. This type of malodor is considered a cosmetic problem and can be treated or masked by several over-the-counter mouth deodorants. The second type of oral malodor involves the proteolytic activity of the microbiota residing on the tongue and tooth surfaces and it is not amenable to masking with deodorants. It is this oral malodor that brings the individual to the dentist and which will be discussed in this review.

We have a superficial knowledge of the biochemical processes that are responsible for the majority, if not all, of this malodor. Namely, there are bacterial types or species living in the mouth which degrade proteins, peptides and amino acids to foul-smelling sulfur compounds, fatty acids and polyamines. We do not know which of the 300 or more bacterial species that live in the mouth are actually involved and what substrate(s) they are degrading. The preponderance of data indicates that the responsible species live on either the tongue, or in the dental plaque, or in both (Frostell and Soder, 1970; Tonzetich, 1977). In this review the most likely microbial contributors to oral malodor will be identified and strategies to control and/or eliminate oral malodor will be described.

II Oral Microbial Ecology

The mouth is resident to over 300 bacterial species, each of which is a specialist in survival. These species cannot all utilize the same nutrients, as this would be a formula for the survival of only the few efficient species. Rather, the complexity of the oral microbiota implies that an extremely diverse nutrient supply is available and that certain organisms base their existence upon their unique ability to degrade, for both energy and biosynthesis, certain nutrients that may be present in minute quantities. These nutrients could come from the diet, especially if the food type is not readily cleared or swallowed, or they could come from the host's own secretions, such as the saliva or gingival crevicular fluid (GCF). They could also come from retention of food on the teeth because of inadequate oral hygiene, or between the teeth because of faulty contact points.

An increase in nutrient supplies from any one of these sources would select for only those species that can metabolize the diverse array of chemicals that are actually present in the source. If a piece of meat is retained between the teeth, bacterial types that can degrade and utilize the various proteins that are present within that particular type of meat will multiply. If some of the released peptides cannot be used by these initial organisms, a second array of organisms will be selected for and increase in numbers. If the food retention causes enough of a population explosion to elicit an inflammatory response from the adjacent gingiva, then still another wave of microbial growth may occur, but this time the bloom will consist of organisms which specialize in degrading the constituents of the gingival crevicular fluid. These organisms may persist if they can maintain the tissue inflammation, in a process which we recognize clinically as gingivitis or periodontitis.

This scenario, while hypothetical, represents a plausible sequence of events that can lead to periodontal disease. It is relevant to our discussion, because each wave of microbial overgrowth in response to nutrient availability would give rise to the volatile sulfur compounds (VSC), such as hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH), and to the volatile fatty acids, such as butyrate, propionate, valerate, etc. which humans perceive as foul smelling. This scenario also implies that where there is oral malodor, there is periodontal disease, or at the very least, poor oral hygiene. There is evidence that associates periodontal disease with oral malodors (Frostell and Soder, 1970; Sato *et al.*, 1980; Yaegaki and Sanada, 1992). Over 80 species, that can be isolated from subgingival plaque, can, *in vitro*, produce either VSC (Persson *et al.*, 1989; 1990), fatty acids, or both. But there is more compelling evidence that the tongue harbors the microbiota that are mainly responsible for oral malodors (Tonzetich, 1977; Bosy *et al.*, 1994). We have no idea which of the many bacterial species that reside on the tongue are responsible, but they may be the same species as are found in the subgingival plaque of periodontal patients. Accordingly, we will describe some of these periodontal species and their metabolic activity against a variety of substrates or nutrients.

III The Malodorous Microbiota

Most of the bacterial species which reside on the oral surfaces are saccharolytic in that they use carbohydrates as their energy source. Other species are asaccharolytic in that they use proteins, peptides, or amino acids as their energy sources, whereas some others, such as *Prevotella intermedia* or *Fusobacterium nucleatum* can use both carbohydrates and proteins (peptides) for energy. The asaccharolytic species, when grown *in vitro*, are malodorous, and because of this, these species are presumed to contribute to oral malodor *in vivo*. Many of these asaccharolytic species are present in subgingival plaque samples, and some, such as the *Porphyromonas* and *Treponema* species, have been extensively studied because of their association with periodontal disease.

Prominent among the putative malodorous species are the large group of organisms formerly called "black-pigmented bacteroides", but now classified as members of the genera *Porphyromonas* and *Prevotella*. Species belonging to these genera, as well as *Treponema, Fusobacterium, Selenomonas, Peptococcus* and *Eubacterium* produce large amounts of short chain fatty acids, such as propionate, butyrate, isobutyrate, valerate, and isovalerate as end products of their metabolism of proteins and peptides. These fatty acids are mainly responsible for the foul smell of laboratory grown cultures of these organisms. Yet, *in vivo* oral malodor is mostly due to the microbes producing VSC from proteins and peptides containing cysteine and methionine (Tonzetich, 1977; Schmidt *et al.*, 1978).

The discrepancy between *in vitro* and *in vivo* types of malodorants can be explained by the abundance of fermentable peptides *in vitro* in the culture media, compared with the low levels found *in vivo* in the saliva or GCF. It is also probable that the levels of the sulfur-containing amino acids, cysteine and methionine, are lower in these media relative to their proportions in peptides that the microbes encounter *in vivo*. Most asaccharolytic species can degrade cysteine and methionine giving rise to fatty acids, H_2S and CH_3SH as end products (Persson *et al.*, 1989). As many as 82 oral species have been shown to produce H_2S from cysteine and 25 species were capable of forming CH_3SH from methionine (Persson *et al.*, 1990).

Cysteine and methionine, as free amino acids, would be present in very small amounts *in vivo*, but could be present in higher levels when incorporated into proteins and peptides. Persson and his colleagues (1990) used serum proteins to simulate the types of substrates that might be available to the plaque microbiota bathed by the GCF. They found 43 species capable of forming H_2S from these serum proteins with *P. intermedia, P. loescheii*, *P. gingivalis, P. endodontalis* and *T. denticola* forming more than 200 µM H_2S (Table 1). Twenty species formed CH_3SH, with *T. denticola* producing 10-20 µM, and *P. gingivalis* and *P. endodontalis* producing 100-200 µM H_2S.

A more detailed presentation of their findings showing the most active species degrading cysteine, methionine or serum is given in Table 2. Those species most active against serum proteins (with the exception of *P. endodontalis*, which produced CH_3SH from methionine) were not particularly active against the free amino acids. As the serum proteins are more likely to represent the types of substrates that these species would find *in vivo*, the five species most active on the serum proteins should be considered as the prime species responsible for the malodor associated with periodontal disease. This malodor reflects primarily the production of CH_3SH relative to H_2S (Table 3) so that *T. denticola, P. gingivalis* and *P. endodontalis* would be particularly suspect. *P. endodontalis* can be removed from this list as it is rarely found in periodontal-associated plaque being, as its name implies, an organism primarily isolated from infected pulps (van Steenberger *et al.*, 1989).

A. The Periodontal Microbiota

If oral malodor is due to periodontal disease, then *T. denticola* and *P. gingivalis* should be prime candidates for the malodorous microbiota. While there is a correlation between VSC in mouth air and the extent of periodontal disease (Tonzetich and Spouge, 1979; Yaegaki and Sanada, 1992), most studies indicate that the majority of oral malodors emanate from the microbiota on the tongue (Tonzetich, 1977; Pitts *et al.*, 1981; Bosy *et al.*, 1994). The importance of the tongue microbiota, even when there is periodontal disease, is illustrated in Table 3. Subjects with periodontal disease had significantly higher levels of VSC in their expired air than did individuals without periodontal disease. If these were all the data collected, then it would seem that the presence of periodontal disease was responsible for the high levels of VSC found. However, Yaegaki and Sanada (1992) also documented the amount of coating material that could be removed from the tongues of their subjects. Individuals with periodontal disease had six times more material

Table 1
Bacterial Species Capable of Producing Either H_2S or CH_3SH from Heat Inactivated Serum

Levels of H_2S	Produced by	Level of CH_3SH	Produced by
1 - 5 μM	13 species	1 - 10 μM	17 species
5 - 20 μM	12 "	10 - 20 μM	*T. denticola*[a]
20 - 100 μM	13 "	100 - 200 μM	*P. gingivalis*[a]
200 μM	*Prevotella intermedia* *Prevotella loescheii* *Porphyromonas endodontalis* *Porphyromonas gingivalis*[a] *Treponema denticola*[a]		*P. endodontalis*

[a]BANA-positive species
Adapted from Persson *et al.*, 1990.

on their tongues, compared with the tongues of the periodontally healthy subjects. As this material was malodorous and would greatly surpass the amount of subgingival plaque that would be present in all the periodontal pockets combined, this tongue coating was probably responsible for the majority of the VSC found in the mouth air. This conclusion is supported by the data of Bosy *et al.*, (1994), which showed no association between the presence of periodontal disease and either mouth odor or VSC in mouth air. In their study the tongue microbiota accounted for all, if not the majority, of the VSC found in mouth air.

B. The Tongue Microbiota

Very little is known concerning the bacterial microbiota indigenous to the tongue. A single study published in 1966 described the total cultivable tongue microbiota of only six subjects (Gordon and Gibbons, 1966). This was prior to the introduction of modern anaerobic culturing techniques and current taxonomic schemes for the identification of oral bacterial species.

Table 2
Most Active Producers of Volatile Sulfur Compounds *in Vitro*

H_2S from cysteine	CH_3SH from methionine	H_2S from serum	CH_3SH from serum
Peptostreptococcus anaerobius *Micros prevotii* *Eubacterium limosum* Unspeciated *Bacteroides* sp. *Centipeda periodontii* *Selenomonad artemidis*	*Fusobacterium nucleatum* *Porphyromonas endodontatis* *F. periodonticum* *Eubacterium sp.* *Bacteroides sp.*	*Prevotella intermedia* *P. loescheii* *P. endodontalis* *P. gingivalis* [a] *T. denticola* [a]	*T. denticola* *P. gingivalis* *P. endodontatis*

[a]BANA-positive species
Adapted from Persson *et al.,* 1990.

Yet, if we refer to the Persson *et al.* (1990) survey of malodorous bacterial species, Gordon and Gibbons (1966) were able to identify potentially malodorous bacteroides, fusobacteria, peptococcus and peptostreptococcus among the prominent cultivable tongue microbiota. Twenty years later, some of these same species were isolated from both the tongue and tonsils (van Winkelhoff *et al.,* 1986). In this latter study, there was the suggestion that the isolation frequency of *P. intermedia*, motile organisms and spirochetes from the tongue tended to be higher if periodontal disease was present.

These two studies were not concerned with identifying the malodorous microbiota of the tongue, and indeed, the studies were performed on subjects who did not complain of oral malodor. Yet, they suggest that the microbiota resident on the tongue resemble the malodorous microbiota found in subgingival plaque. We have examined the tongue microbiota of 16 individuals who presented with self-reported complaints of malodor and who had an organoleptic score of 2.9 (clearly noticeable malodor of moderate intensity). We used an anaerobic chamber procedure and a variety of selective media for the purposes of identifying whether the malodorous microbiota could be

Table 3
Production of VSC by the Tongue Microbiota in Subjects With and Without Periodontal Disease

Periodontal Disease[a]	**Wet Weight of Tongue Coating (mg)**	**VSC ng/10 ml**	**CH_3SH/H_2S**
No (14)[b]	14.6 ± 7.5	4.3 ± 3.1	1.0 ± 0.3
Yes (17)	90.1 ± 11	18.6 ± 6.8	31.3 ± 18.1

[a] Subjects with disease had probing depths $\geq$ 4mm
[b] Number of subjects in each group
Adapted from Yaegaki and Sanada (1992).

associated with the anaerobic and asaccharolytic microbiota, as opposed to the facultative and saccharolytic microbiota.

We also examined for the presence of several malodorous subgingival plaque species and for the presence of species capable of hydrolyzing the synthetic trypsin-like substrate benzoyl-DL-arginine naphthylamide (BANA). *P. gingivalis* and *T. denticola*, two of the most malodorous species when tested *in vitro* on serum proteins (Tables 1 and 2), both possess a BANA hydrolytic enzyme (Loesche *et al.*, 1985; Loesche, 1986), which can be easily measured using a commercially available kit (BANAScan, Knowell Therapeutic Technologies, Toronto, Canada). Thus, a positive BANA test for the tongue might indicate the presence of these or other malodorous species which may also possess the BANA enzyme. The combination of a BANA-positive reaction and high mouth VSC have been shown by Kozlovsky *et al.* (1994) to give the best correlation with the organoleptic scores of mouth air.

No putative malodorous species could be found in every tongue sample. *Fusobacterium* sp. (this would include *F. nucleatum, F. fusiform* and *F. polymorphum*), *Capnocytophaga* species, *Rothia dentocariosa*, and *P. intermedia* were found in 56 to 70% of the samples (Table 4). The known cultivable BANA-positive species, with the exception of *Rothia dentocariosa*, were not prominent among the identified isolates. However, only 28 to 30% of the cultivable microbiota could be identified to the species or genus level with the methods employed. This inability to easily identify the majority of the cultivable microbiota has long been a methodological problem in mucous membrane microbiology, given the vast number of species present, and the fact that most species, including those on the tongue, have yet to be classified.

Our strategy to deal with this problem was to divide the microbiota into segments which corresponded to known physiologic states. We knew that the majority of the malodorous microbiota were anaerobes and that they were proteolytic (Persson *et al.,* 1990). Accordingly, we incubated our inoculated bacteriological media under anaerobic conditions that would allow only facultive species to grow (by incubating anaerobically on media containing 20 μg/l metronidazole; Loesche *et al.,* 1985), or in air which would allow aerobic organisms to grow. We used a medium that was free of carbohydrates to detect the asaccharolytic microbiota. We also directly measured the BANA reactivity of tongue samples, in case difficult-to-cultivate species, such as *T. denticola* and *B. forsythus*, or possibly some uncultivable BANA-positive species were present (Bosy *et al.,* 1994).

This strategy was used to monitor bacteriological changes in the tongue before and after treatment for the malodor. The results of this investigation will be reported elsewhere (De Boever and Loesche, submitted), and will be briefly summarized. Sixteen subjects who reported having oral malodor, confirmed by a subjective organoleptic score and elevated VSC in mouth air (Rosenberg *et al.,* 1992; De Boever *et al.,* 1994), were instructed to rinse twice daily for a period of one week with a solution of 0.12% chlorhexidine gluconate (Peridex™, Procter and Gamble) and to brush their tongue with a toothbrush dipped in the chlorhexidine gluconate.

This treatment resulted in a significant reduction in the organoleptic score and in the VSC levels present in mouth air (Table 5). Among the bacteriological parameters there was a significant reduction in the percentages of tongue samples that were BANA-positive, a reduction in the anaerobic microbiota as measured by a reduced anaerobic/aerobic ratio, but no reduction in the asaccharolytic microbiota (Table 5). The reduction in the percentage of BANA-positive tongue samples is consistent with the findings of Bosy *et al.* (1994), who found that a 7-day of rinsing with chlorhexidine significantly reduced the tongue BANA scores. These reductions suggested that the BANA-positive plaque species identified as producing VSC *in vitro* from serum proteins, *i.e.*, *P. gingivalis* and *T. denticola* (Persson *et al.,* 1990), are either

Table 4
The Presence of Malodorous Species in Coating Material Removed from the Tongues of Individuals with Complaints of Oral Malodor

Species	Prevalence	% of Viable Count	BANA Status[a]
P. gingivalis	1/16	0.02	+
P. intermedia	9/16	0.44	-
P. melaninogenica	0/16	0	-
Fusobacterium sp.	11/16	2.53	-
Capnocytophaga sp.	11/16	1.97	-
Capnocytophaga gingivalis	4/16	0.6	+/-
Rothia denticariosa	10/16	1.5	+

[a] The *in vitro* BANA reaction of these species (see Loesche *et al.*, 1985)

responsible for oral malodors emanating from the tongue, or biochemically resemble the species that are actually producing the malodor.

The Persson *et al.* (1990) study suggested that serum proteins present in the GCF would be the substrate(s) used by these organisms to produce the VSC. The GCF would not be available to the tongue microbiota, as it would be diluted greatly in the saliva. However, the saliva itself contains a rich source of glycoproteins and aminoglycans that would contain cysteine and methionine. When saliva is allowed to incubate *in vitro*, it putrefies as certain Gram-negative species overgrow (McNamara *et al.*, 1972).

IV Treatment Strategies

If the bacterial microbiota on the tongue and in the dental plaque are the main reservoirs for the malodorous microbiota, then treatment should focus on the reduction of the levels, if not the elimination of these organisms, from these sites (Rosenberg, 1990). The treatment can be directed solely towards the bacteria, but a more comprehensive approach would address the nutrient availability as well.

Table 5
Changes in Selected Bacteriological Parameters in Tongue Samples Removed from Before and After Treatment for Oral Malodor[a]

Parameter under investigation	Treatment Before	 After	Significance (paired t-test)
Organoleptic Score	2.9	0.9	< 0.001
Volatile Sulfur Compounds (ppb)	309	82	0.001
% BANA-positive	69	19	0.003
Anaerobic/Aerobic Ratio	2.29	1.46	

[a] Data taken from De Boever and Loesche, 1995

A. Reduction of Nutrient Availability

There are almost no scientific data on the role of nutrient availability in oral malodor. If the nutrients are derived from the GCF because of periodontal disease, or from food retention because of poor interproximal contact points, then these sources need to be eliminated by periodontal treatment and operative dental treatment that improves the self-cleansing contours on the tooth surfaces. The individual's eating habits should be examined to determine if certain dietary features, *i.e.*, a high protein diet, or frequent eating may be associated with the malodor. A low carbohydrate, high protein diet that was recommended for caries control more than 60 years ago was associated with complaints of oral malodor (Philip Jay, personal communication). It may be that such a diet increases the amount of proteins, peptides and amino acids in the saliva, thereby selecting for a malodorous microbiota on the tongue.

The role of saliva as a source of nutrients capable of being fermented to VSC and volatile fatty acids is not known. One can envision a scenario in which either too little or too much saliva could contribute to oral malodor. If there is too little saliva, then the microbial density of the saliva increases, and the contact time between the tongue microbiota and the various sulfur containing compounds in the saliva would increase secondary to the reduced swallowing that is associated with a low salivary flow (Lear, 1970). Both of these situations, an increased microbial load and an increased microbial contact time with substrates, should augment the amount of VSC and fatty acids produced by the tongue microbiota. However, salivary flow that is stimulated by frequent eating or chewing could also contribute to oral malodors by bringing more nutrients to the tongue microbiota. These nutrients would stimulate bacterial growth on the tongue and could explain the heavy coatings of bacteria often seen on the tongues of individuals who complain of malodor (see Table 3).

These two scenarios involving low salivary flow and frequent stimulation of saliva flow illustrate both the critical role of saliva in oral malodor and our ignorance of precisely what that role is. Information in this regard would be necessary in order to definitively treat oral malodors. Until this is available treatments will have to be based against the bacterial component.

B. Reduction of the Bacterial Load

Treatments aimed at reducing the bacterial load on the teeth and tongue would be an obvious and necessary approach to controlling oral malodors. If the subject has poor oral hygiene, periodontal disease or dental caries, the treatment of these conditions has the highest priority. In conjunction with the normal hygiene instructions, the individual should be instructed to debride the surface coating from the tongue (Rosenberg, 1990; Bosy *et al.*, 1994). This can be accomplished by scraping the tongue surface with a pliable plastic or wooden stick. The stick would be placed at the rear of the tongue and pulled forward to remove the surface coating. This procedure would be repeated several times until very little coating material can be recovered by the stick.

Alternatively, the individual could brush his tongue with a regular toothbrush. In the study shown in Table 5, the subjects brushed their tongues with a toothbrush dipped in chlorhexidine gluconate after using a chlorhexidine rinse. This regimen resulted in a prompt reduction in VSC and in the organoleptic score, which coincided with a decrease in BANA-positive organisms, anaerobes, and asaccharolytic organisms in the tongue scrapings. This study design would not allow the determination of how much of this improvement was due to the mechanical debridement and how much was due to the chlorhexidine.

The results encourage research into development of antimicrobial agents which can be used to control for the microbiota that contributes to oral malodor. This is because tongue debridement to some individuals is an onerous chore, and one which they would replace if an effective antimicrobial agent were available. The bitter taste of chlorhexidine discourages its use and its long-term effects on taste perception and mucous membranes preclude its usage on a continuing basis (Flotra, 1971). Rosenberg *et al.* (1992) have described short-term reductions of oral malodor that were comparable to those obtained using chlorhexidine, using a two-phase oil and water mouthrinse. Further studies are needed to determine whether such an agent can replace chlorhexidine.

Acknowledgments

This research was supported in part by NIDR grant DE09142, and in part by a gift from Oral-B Laboratories.

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4

Advances In Mouth Odor Research

Hollandra P. Niles* and Abdul Gaffa

Local mouth odor is caused by oral bacterial reacting with salivary proteins to form volatile sulfur compounds (VSC). Tonzetich (1977) has shown that hydrogen sulfide, methyl mercaptan and dimethyl sulfide (H_2S, CH_3SH and $(CH_3)_2S$, respectively) are the major components of mouth odor. A gas chromatographic method was developed to objectively measure VSC directly from mouth air as an alternative to the organoleptic/sensory method. This instrumental method has in turn permitted investigators to carry out studies in a number of areas relevant to human oral malodor. In this chapter we discuss (i) the role of microorganisms in oral malodor; (ii) the relationship between oral malodor and gingivitis; (iii) the correlation between sensory and instrumental evaluation of oral malodor; and (iv) approaches to oral malodor control.

The Role of Microorganisms

Oral microorganisms play a role in the production of intrinsic malodor. In the absence of microorganisms the odoriferous components are not generated. McNamara and colleagues (1972) determined by an *in vitro* method that Gram-negative, rather than Gram-positive organisms, were responsible

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for the production of the odor components. However, they did not identify the nature of the components.

Therefore, a separate study was conducted (Solis-Gaffar *et al.,* 1979) to determine the role of specific Gram-positive and Gram-negative organisms responsible for VSC formation. This determination was made by cultivating seven Gram-positive and four Gram-negative organisms which represent the predominant microbiota in the oral cavity. Socransky and Manganiello (1971) found that different organisms colonize different oral sites. The predominant sites for Gram-negatives are the gingival crevice, interdental spaces and dental plaque. *Prevotella melaninogenica* and *Fusobacterium nucleatum* are predominant in the gingival crevice, while *Veillonella alcalescens* is found in plaque and *Klebsiella pneumoniae* in the interdental spaces. These organisms were incubated anaerobically in a sterile system with L-cysteine in the presence of human blood. After incubation, the head space was evaluated organoleptically and instrumentally. Controls consisted of sterile saliva, sterile saliva/L-cysteine, L-cysteine/H_2O and non-sterile saliva/L-cysteine.

The results (Table 1) indicate that only the Gram-negative organisms are capable of producing VSC. Of the controls tested, only the non-sterile saliva/L-cysteine control produced VSC and malodor; the other controls did not form VSC or noticeable malodor.

pH changes were also evaluated on each sample before and after incubation. The Gram-positive organisms did not show any appreciable change in pH, whereas the Gram-negatives showed a noticeable alkaline shift, in particular with *Klebsiella pneumoniae* (Table 2). Similarly, McNamara and colleagues previously determined that there was a shift in the ratio of Gram-positives to Gram-negatives favoring Gram-negatives during saliva stagnation and also observed a simultaneous alkaline pH shift.

This study confirmed that Gram-negative anaerobes which are found at sites of saliva stagnation in the mouth are responsible for VSC production in sterile saliva. The VSC production is accompanied by a shift to alkaline pH. The specific Gram-negative microorganisms identified involved in VSC production are *Prevotella melaninogenica, Fusobacterium nucleatum, Veillonella alcalescens* and *Klebsiella pneumoniae.*

The Relationship Between Oral Malodor and Gingivitis

Hydrogen sulfide is a toxic metabolite that has been detected in the gingival

Table 1
Odor and VSC Production by Organisms

Organisms	VSC detected	Odor produced	Gram stain
Streptococcus sanguis	NO	NONE	POSITIVE
Streptococcus salivarius	NO	NONE	POSITIVE
Streptococcus mutans	NO	NONE	POSITIVE
Actinomyces naeslundii	NO	NONE	POSITIVE
Lactobacillus acidophilus	NO	NONE	POSITIVE
Staphylococcus aureus	NO	NONE	POSITIVE
Candida albicans	NO	NONE	–
Prevotella melaninogenica	YES	UNPLEASANT	NEGATIVE
Fusobacterium nucleatum	YES	UNPLEASANT	NEGATIVE
Veillonella alcalescens	YES	UNPLEASANT	NEGATIVE
Klebsiella pneumoniae	YES	UNPLEASANT	NEGATIVE

sulcus by Rizzo (1967) and Horowitz and Folke (1973) using a lead acetate method. Since there is an apparent relationship between the volume of gingival crevicular fluid (GCF) and the progression of periodontal disease, a method was developed to quantify H_2S production by GCF. This methodology in turn allowed the study of the relation between hydrogen sulfide production in GCF and gingival health (Solis-Gaffar *et al.*, 1980).

The study design required 20 male subjects to be evaluated using a modified Löe and Silness (1963) Gingival Index (GI). Male subjects were used to minimize the influence of hormonal changes on the GCF flow rate. Subjects were given multiple prophylaxis treatments to bring the baseline GI to 0.67. Evaluations of GI and H_2S levels in GCF were conducted at baseline and at one week intervals for 4 weeks.

Two GCF samples were obtained from each subject using the gingival crevices mesial to the six anterior maxillary teeth. A sterile paper strip was inserted into each site for 3 seconds to remove any initial fluid present and,

Table 2
pH Changes Before and After Incubation at 37°C

Organisms	**pH before incubation**	**pH after incubation 3 h**	**24 h**
Klebsiella pneumoniae	7.6	10.0	10.5
Fusobacterium nucleatum	7.2	7.2	7.9
Prevotella melaninogenica	7.0	7.7	8.9
Veillonella alcalescens	7.2	7.3	7.5

after 30 seconds, a second strip was inserted for 3 seconds. The volume (µl) of crevicular fluid in the second strip was measured with a Periotron/GCF Meter. The H_2S determinations were made from the filter strips by incubating each strip with L-cysteine in 2 ml sterile saliva. The headspace was then evaluated for the levels of H_2S by gas chromatograph. Spearman's rank correlation was computed to determine the relationship between the mean GI value, GCF volume and amount of H_2S generated.

The results are depicted graphically (Figure 1). The y-axis units are in microliters (µl) of crevicular fluid and nanograms (ngs) of H_2S. The gingival index is shown on the horizontal axis starting with the baseline GI level of 0.67.

Positive but moderate correlations were observed between the GI and the volume of GCF ($r = 0.53$) and between GI and the H_2S production by GCF ($r = 0.41$). An improved correlation was observed between the H_2S levels generated and GCF volume ($r = 0.66$). As the volume of crevicular fluid increased the levels of H_2S generated also increased.

The results demonstrated a good correlation between GCF volume and H_2S production. The H_2S generating potential of GCF was also found to increase with the severity of gingival inflammation. H_2S production by GCF could therefore be useful in monitoring the initial stages and severity of gingivitis and periodontitis.

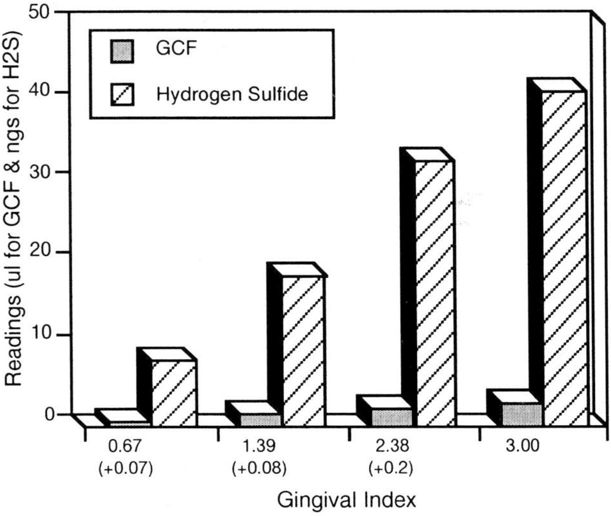

Figure 1. Relationship between GI, GCF and hydrogen sulfide.

The gas chromatograph (GC) technique was used to demonstrate (i) the relationship between Gram-negative organisms and VSC production; and (ii) a correlation between the VSC production in crevicular fluid and degree of inflammation.

The Correlation Between the Organoleptic and Gas Chromatograph Methods for Assessing Oral Malodor

There are two methods currently available to assess the magnitude of oral malodor. The first is the organoleptic or sensory rating approach and the second is the GC instrumental method (Solis-Gaffar *et al.*, 1975; Richter and Tonzetich, 1964). A study was conducted to determine the correlation between these two methods in a controlled clinical study (Niles and Gaffar, 1993).

Twenty subjects qualified to participate in the study by meeting the inclusion/exclusion criteria of the protocol. Mouth air samples were collected in storage loops and transported to the laboratory for later analysis (Figure 2). Sensory evaluations were conducted by two professional judges at the clinical site following collection of the mouth air samples.

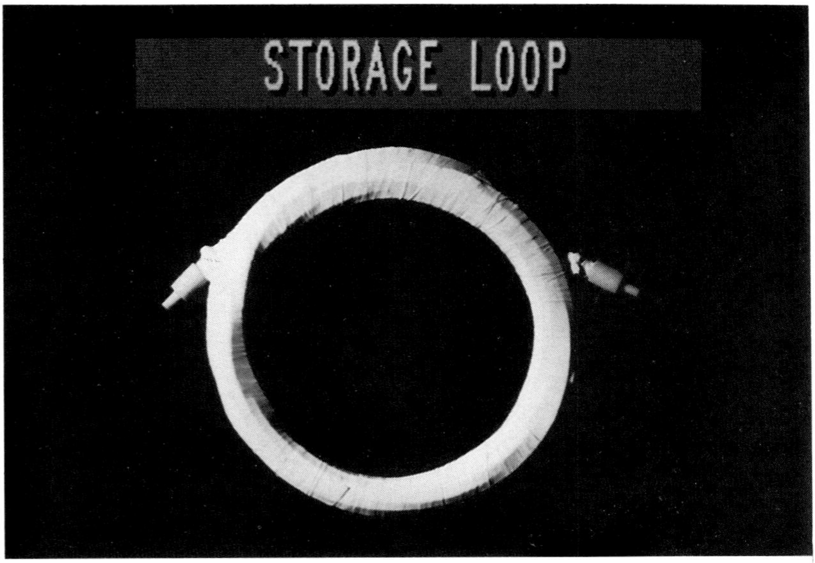

Figure 2. Teflon storage tube.

The storage loop in which mouth air samples were collected consisted of 7 meters of coiled Teflon tubing, with a 1/8 inch outer diameter and 1/16 inch inside diameter wrapped in Teflon tape. A mini-nert valve was attached to each end of the tubing. Each loop was calibrated to hold 10 cc of mouth air. A 50 cc syringe was attached to one end of the tubing while a sterile Teflon tube was attached to the other end. After sitting for 10 min with their mouth closed, the tube was carefully inserted into the subjects' mouth between their closed lips. With both mini-nert valves opened, the mouth air was drawn into the storage loop via the syringe. The valves were closed and the stored samples were transported to the laboratory in dry ice where they were evaluated within 5 to 7 h of collection.

The second method for the evaluation of mouth air involved two professional judges rating the offensiveness of mouth air on a 0-8 scale. A rating of 0 represents the non-offensive level and 8 represents a highly offensive level (Table 3). Two evaluations were conducted by each judge. Subjects were asked to sit with their mouths closed for 10 min prior to each evaluation.

Table 3
Sensory Evaluation Criteria for Mouth Odor

Sensory Score	Offensiveness of mouth odor
8	Strong
7	
6	Definite
5	
4	Faint
3	
2	Doubtful
1	
0	None

The study consisted of three phases: control, test and recovery. Each phase was 3 days in duration and evaluations of oral malodor were performed on the second and third day of each phase. Subjects practiced their usual oral hygiene during the control and recovery phases. During the test phase, subjects were instructed to brush for 1 min with a placebo dentifrice in the morning and at night for 2 days (day 1 and 2 of the test phase).

The results showed a poor correlation between the two methods during the initial control phase with a correlation coefficient of 0.22. This poor correlation was attributed to the lack of familiarity of the subjects with the sampling procedures.

During the test and recovery phases the correlation improved with correlation coefficient values of 0.77 and 0.78, respectively (Table 4). The results indicate a good correlation between the instrumental and organoleptic methods for the evaluation of mouth odor. The correlation improved as the subjects adjusted to the sampling procedure. Both methods are beneficial for measuring intensity of offensiveness of mouth air.

Table 4
Mean Instrumental Values Vs. Mean Sensory Ratings

Determination	N	Control period		Test period		Recovery period	
		Day 2	Day 3	Day 2	Day 3	Day 2	Day 3
Sensory Rating	20	7.15	6.98	6.43	6.45	6.38	6.55
		±0.42[a]	±0.76	±0.73	±0.82	±0.70	±0.69
Volatile Sulfur	20	21.86	22.77	20.14	20.16	23.12	22.22
ngs/ml		±4.18	±6.00	±6.12	±4.97	±6.13	±4.06
Correlation (r) Coefficient		0.22 (NS)[b]		0.77 ($p < 0.01$)		0.78 ($p < 0.01$)	

[a] Mean ± Standard Deviation
[b] Not significant

Therefore, the instrumental method has since been established as a useful tool for the *in vitro* and human clinical evaluation of VSC.

Approaches to Oral Malodor Control

There are many products in the commercial market with mouth odor control claims and these products attempt to control oral malodor using one or a number of different approaches. A segment of the commercial products uses a masking approach where the product flavor and corresponding scent masks oral malodor. Alternatively, an antibacterial approach incorporates active ingredients into the product which attempt to reduce the formation of VSC by controlling the growth or metabolism of Gram-negative microorganisms in the oral cavity. Tonzetich (1977) noted that in order for a compound to be odorous it must be volatile. Therefore, the VSC conversion to non-volatiles is an approach for reducing malodor by converting the odorous volatile acidic components to non-volatile, non-odorous forms.

The latter two approaches have been investigated in human clinical studies. Specifically, the antibacterial approach was examined with the use of actives such as cetylpyridium chloride (CPC), benzethonium chloride (BTC) and

Table 5
Three Hour Effect of a CPC Rinse on VSC

Treatment	N	Initial[a]	Final[a]	% Inhibition	p-value
CPC Rinse	16	15.70 (±2.32)	10.93 (±3.13)	30.39[b]	(p=0.002)
Placebo Rinse	16	15.13 (±3.68)	13.03 (±4.03)	13.88	(p=0.002)

[a] Mean ± Standard Deviation (ng/ml total sulfur)
[b] Significance between rinses: p = 0.006

phenolic flavor oils. The volatile acid conversion approach was examined clinically with the use of zinc or magnesium salts, or sodium bicarbonate.

These clinical studies determined the short-term (3 h) effect of an active ingredient by (i) comparing the mean VSC values at baseline (initial) and after use of the test product (final); and (ii) comparing the final mean VSC values of test and placebo products.

Antibacterial Approach

i. Cetylpyridinium chloride (CPC)

The GC method of sulfide evaluation involves the quantitative analysis of the major components of mouth odor. The first study evaluated the short term (3 h) effect of CPC compared to a placebo rinse. A 30% statistically significant inhibition was observed with CPC compared to the initial mean (Table 5). Also, when the final means for the two products were compared, the CPC rinse was superior to the placebo rinse in reducing VSC production at 3 hours post-treatment (p = 0.006).

Table 6
Three Hour Effect of BTC

H_2S Reduction

Treatment	Initial[a]	Final[a]	%Inhibition
Placebo	27.93	27.27	2.36%
BTC	27.03	23.01	14.87[b]

CH_3SH Reduction

Treatment	Initial[a]	Final[a]	% Inhibition
Placebo	28.03	27.45	2.01%
BTC	27.45	16.27	40.73%[b]

[a] Mean values (ng/ml total sulfur)

[b] Significance between treatments $p < 0.05$

ii. Benzethonium chloride (BTC)

Another study evaluated the effects of BTC. Levels of the two major components, H_2S and CH_3SH, were determined and reported separately in this study. The results showed that BTC was active in reducing CH_3SH by 40% versus the initial mean baseline value, whereas H_2S showed a 15% reduction (Table 6). Statistical significance between the BTC and placebo rinses was also observed ($p < 0.05$).

iii. Phenolic flavor oils

Phenolic flavor oils in general and thymol and eugenol in particular, are known to have an antibacterial effect. A significant clinical effect was observed with the phenolic flavor oils providing a 45% reduction in VSC versus mean baseline levels (Table 7).

Table 7
Four Hour Effect of Phenolic Flavor Oils on VSC

Treatment	Initial[a]	Final[a]	% Inhibition	p-value
Flavor Oils (Phenolics)	14.28[a] (±2.25)	7.84[b] (±3.90)	45.10	(p=0.0001)
CPC	13.63 (±2.65)	9.95[b] (±4.88)	27.00	(p=0.002)

[a] Mean ± Standard Deviation (ng/ml total sulfur)
[b] Significance between rinses: p= 0.120

In summary, the antibacterial approach can result in reduced formation of VSC as the clinical use of rinses containing CPC, BTC and the phenolic flavor oils have been demonstrated.

Acid Conversion Approach

As previously mentioned, the odorous acid volatiles responsible for oral malodor can be converted to a non-odorous form. The next two studies evaluated a zinc salt and sodium bicarbonate on VSC reduction.

i. Sodium bicarbonate

A sodium bicarbonate/fluoride dentifrice was compared to a regular fluoride dentifrice in a human clinical study designed to determine the short-term (3 hour effect) of product use. The results (Table 8) indicate that the sodium bicarbonate dentifrice was effective giving a statistically significant 43% reduction versus mean baseline VSC levels ($p < 0.01$). A comparison of the final mean VSC levels provided by the two products indicate that the sodium bicarbonate dentifrice was superior to the fluoride dentifrice ($p = 0.01$).

Table 8
Three Hour Effect of Sodium Bicarbonate Dentrifrices on VSC as Compared to Fluoride Dentrifice

Treatment	N	Initial[a]	Final[a]	% Inhibition
Na Bicarbonate	16	14.86 (±2.44)	8.35[b] (±3.19)	43.8
Fluoride	16	14.82 (±2.21)	10.26[b] (±4.18)	30.77

[a] Mean ± Standard Deviation (ng/ml total sulfur)

[b] p= 0.01 between final treatment means; significance between dentifrices:p=0.01

ii. Zinc chloride

Using the same clinical design as with sodium bicarbonate, the efficacy of zinc chloride ($ZnCl_2$) was examined in a rinse. The zinc ion was effective giving a statistically significant 24% reduction in VSC versus the mean baseline score (Table 9). When the final mean VSC scores were compared, the zinc chloride-containing rinse was also superior to the placebo rinse ($p < 0.01$). A flavor component was selected to enhance this effect, which leads to the combination approach.

Zinc with Alpha-Ionone, a Combined Approach

Folklore in the US indicates that tomato juice is traditionally used to neutralize skunk odor on clothes and pets. Skunk odor results from volatile sulfide components, similar to the components responsible for oral malodor in humans. When tomato juice flavor components were evaluated individually and in combination, it was observed that beta-ionone alone and in combination with a zinc salt strongly inhibited VSC formation *in vitro*. Alpha-ionone, having a more acceptable taste, was substituted for the beta-ionone form.

Table 9
Three Hour Effect of Zinc Chloride Rinse

Treatment	N	Initial mean[a]	Final mean	%Inhibition
0.25% ZnCl$_2$ Rinse	12	13.60 (±2.50)	10.30 (±0.60)	24.0[b]
Placebo Rinse	12	13.50 (±2.70)	11.70 (±1.80)	13.0[b]

[a] Mean ± Standard Deviation VSC (ng/ml total sulfur)

[b] Significance between rinses: p= 0.003

Based on the *in vitro* results, a zinc/alpha-ionone combination was tested clinically resulting in an increased effectiveness with 59% reduction versus the initial baseline mean (Table 10). A statistically significant difference was also observed when the final mean VSC scores for the zinc/alpha-ionone and placebo rinses were compared. Using the results of Table 9, the zinc/alpha-ionone combination was also about twice as effective as zinc chloride alone.

These clinical results indicate that the above approaches, when used alone or in combination, can significantly reduce VSC production, thereby controlling the intensity of offensive local mouth odor for 3-4 hours.

Summary

In summary it has been demonstrated that:

1. Gram-negative organisms are primary contributors to the production of VSC in the local oral cavity and VSC production parallels offensive oral malodor.
2. There is a relationship between H_2S production and gingival inflammation.
3. A significant correlation has been established between the GC instrumental and the organoleptic methods.

Table 10
Three Hour Effect of Consumed Zinc Salts/Alpha-Ionone Rinse

Treatment	N	Initial[a] mean	Final mean	% Inhibition	p-value
0.4% Zn/ 1% alpha-ionone	13	13.92 (±2.49)	5.70[b] (±2.24)	59.06	(p= 0.0001)
Placebo	12	15.65 (±2.44)	13.03[b] (±3.87)	16.75	(p= 0.041)

[a] Mean ± Standard Deviation (ng/ml total sulfur)
[b] Significance between rinses: p= 0.007

4. The GC instrumental method is a useful tool for the objective measurement and evaluation of VSC in both *in vitro* models and human clinical studies.
5. Several treatment approaches exist which can provide clinically detectable and meaningful reductions in local mouth odor for short periods of time (3-4 hours).

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5

Association of Diamines with Oral Malodor

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Oral malodor is a common problem affecting a large proportion of the adult population (Tonzetich, 1973; 1977). In most cases, malodor originates within the oral cavity itself, as a result of microbial putrefaction of host components and food residues. Oral bacteria hydrolyze proteins and further degrade the amino acids, yielding ammonia, volatile sulfur compounds (VSC), indole, lactic acid and other components (Cary, 1946; Rae and Clegg, 1956). Although the microorganisms which cause bad breath *in situ* have not been identified, it is likely that Gram-negative microorganisms are primarily responsible (Niles and Gaffar, this volume; Kleinberg and Codipilly, this volume; Loeche and De Boever, this volume). When inoculated into liquid broth, Gram-negative oral anaerobes, such as fusiforms, spirochetes, vibrios, and bacteroides, produce putrid compounds (McNamara *et al.,* 1972). Bacterial samples from deep periodontal pockets incubated in human serum produce high levels of VSC, especially hydrogen sulfide (Persson *et al.,* 1989). Malodor production *in vitro* is favored not only by anaerobisis but also by basic pH which is associated with hydrolysis of protein (Kleinberg and Codipilly, this volume; Loesche and De Boever, this volume). Incubation of saliva itself results in an increase in putrid odor and in relative numbers of Gram-negative microorganisms.

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However, addition of 20 mM glucose to saliva causes a decrease in pH, inhibition of odor production and a shift towards Gram-positive bacteria (McNamara *et al.,* 1972). Saliva contains about 10 μg/ml free glucose and 150 μg/ml carbohydrate in glycoproteins. Proteins are present in much greater amounts, *i.e.,* 2-3 mg/ml. Proteins are also available in the crevicular fluid and in exfoliated cells from the oral mucosa. Patients with degenerative periodontal diseases tend to have increased numbers of exfoliated cells and bacteria in their mouths, as well as higher malodor levels (Tonzetich and Kestenbaum, 1969; Tonzetich, 1973; Kostelc *et al.,* 1981).

The Chemical Composition of Oral Malodor

Most previous investigations which have addressed the gaseous components contributing to oral malodor have concentrated on volatile sulfur compounds (VSC), primarily hydrogen sulfide and methyl mercaptan (Tonzetich and Richter, 1964; Tonzetich, 1971). Precursors of these compounds include cysteine, cystine and methionine (Kleinberg and Westbay, 1990).

VSC levels, measured directly from the mouth using a portable sulfide monitor, show significant correlations with organoleptic measurement of bad breath (Rosenberg *et al.,* 1991a,b, 1992; Bosy *et al.,* 1994; De Boever *et al.,* 1994). Gas chromatographic analysis of VSC is also associated with oral malodor (Schmidt *et al.,* 1978; Niles and Gaffar, this volume). Relatively high levels of VSC, particularly methyl mercaptan, have been found in mouth air of patients with periodontal diseases (Yaegaki and Sanada, 1992). Moreover, uptake of VSC by epithelial cells may play an important role in the pathogenesis of periodontal disease (Tonzetich, 1978; see also Tonzetich, this volume).

Other Volatile Compounds

Several studies have addressed the molecular composition of bad breath and of the headspace of human saliva using gas chromatography. Some of the volatiles detected are listed in Table 1.

Pyridine and methylpyridines were found in the salivary headspace of patients with periodontal diseases but not in the salivary headspace of healthy subjects. Elevated levels of VSC, phenol, cresol, dodecanol, tetradecanol, hexadecanol, indole and skatole were also found in the salivary headspace of patients with periodontal disease (Kostelc *etal.,*1980). In a study by Tonzetich

Table 1
Malodorous Compounds Found in Human Saliva or in Salivary Headspace

Compound	Found in normal subjects	Elevated in subjects with periodontal disease	Reference
hydrogen sulfide	+	+	Yaegaki and Sanada, 1992
methyl mercaptan	+	+	Yaegaki and Sanada, 1992
dimethylsulfide	+	+	Tonzetich *et al.*, 1978
indole	+	+	Kostelc, 1981
skatole	+	+	Kostelc, 1981
lactic acid	+		Rae and Clegg, 1956
methylamine	+	+	Fosdick *et al.*, 1953
diphenylamine	+	+	Kostelc, 1981
cadaverine	+		Fosdick *et al.*, 1953
putrescine	+		Fosdick *et al.*, 1953
urea	+		Golub *et al.*, 1971
ammonia	+		Golub and Kleinberg, 1976
dodecanol	+	+	Kostelc *et al.*, 1980
tetradecanol	+	+	Kostelc *et al.*, 1980
hexadecanol	+	+	Kostelc *et al.*, 1980
phenol	+	+	Kostelc, 1981
cresol	+	+	Kostelc *et al.*, 1980
pyridine	–	+	Kostelc *et al.*, 1980
δ-aminovaleric acid	+	+	Fosdick and Piez, 1953
methylpyridines	–	+	Kostelc *et al.*, 1980

and coworkers (1967), the potential contribution of nonsulfur components to the oral halitus was challenged. Indole, methylamine and cadaverine were added to bulk saliva with no subsequent increase in odor, nor detection of these components in the headspace. The authors concluded that such compounds were not involved in oral malodor. However, oral malodor increases with drying of oral surfaces, suggesting that such components are released into the oral headspace when saliva dries out on the mucosal surfaces (Rosenberg and McCulloch, 1992). When solutions of indole, skatole, putrescine, cadaverine, butyric acid and valeric acid were allowed to dry on human skin, prolonged release of odor was observed (Kleinberg and Codapilly, this volume).

Diamines

Cadaverine and putrescine are malodorous diamines, commonly encountered as bacterial degradation products. Cadaverine may be produced in saliva as the result of decarboxylation of lysine (Gale, 1946). Putrescine may be produced by (i) decarboxylation of ornithine (Gale, 1946); (ii) decarboxylation of arginine, resulting in agmatine which is subsequently converted to putrescine; (iii) deimination of arginine, resulting in citrulline, which is converted to putrescine via ornithine; or (iv) direct conversion of arginine to ornithine, followed by decarboxylation of ornithine to putrescine. Cadaverine and putrescine are found in human dental plaque (Hayes and Hyatt, 1974). Decarboxylation of amino acids is generally considered to be a strategy of some bacteria to counter unfavourable acidic growth conditions produced during glycolysis (Gale, 1946). Decarboxylases were found to be active at low pH values characteristic of dental plaque (4-6), and showed substrate specificity (Gale, 1946; Hayes and Hyatt, 1974).

The present chapter examines the potential role of cadaverine and putrescine as components of oral malodor, based on three different experimental approaches. Initially, a clinical study was conducted, in which dental and oral malodor parameters were compared to diamine levels in the saliva of 52 volunteers. In order to test whether these diamines are also produced *in vitro,* experiments were conducted employing saliva samples inoculated into decarboxylase medium. In the third approach, paper points taken from deep and shallow periodontal pockets of 24 patients were inoculated into decarboxylase medium. Odor elaboration, pH and diamine production were followed over time.

Clinical study

Details of the clinical study reported here have been recently published (Kozlovsky *et al.,* 1994; Goldberg *et al.,* 1994), and are described here in brief. Fifty-two subjects (35 females; mean age 37), 43 of whom complained of bad breath, participated. Parameters included measurement of intraoral VSC using a portable industrial sulfide monitor (model #1170, Interscan

Corp. Chatsworth, CA), connected to a pen recorder, odor judge scores, Plaque Index (PI), Gingival Index (GI), mean probing depth, trypsin-like activity (BANA test), and salivary levels of cadaverine and putrescine.

For measurement of diamine levels, high performance liquid chromatography (HPLC) analysis was employed (Goldberg *et al.*, 1994). Saliva samples were first derivatized using O-phthaldialdehyde reagent and then injected into the HPLC system. Peak and steady-state VSC measurements were made using the sulfide monitor. Whole mouth, tongue and saliva malodors were recorded on a 10 cm visual analog scale by an experienced odor judge. Whole mouth malodor was measured as described previously (Rosenberg *et al.*, 1991a,b). Tongue malodor was measured by instructing subjects to lick their wrist, and smelling it at a distance of *ca.* 3 cm following five seconds. Saliva samples, obtained following the dental examination, were allowed to stand at 37°C in a closed Petri dish for 5 minutes and were then smelled at a distance of *ca.* 5 cm. The BANA test was performed using samples obtained from four loci: shallow pockets, deep pockets, tongue and saliva.

Cadaverine levels in the 52 saliva samples tested ranged from 0 to 1395 μM, with a mean of 371.6 ± 307.6 μM (SD). Putrescine concentrations averaged 109.0 ±92.7 μM , ranging from 21.4 to 599.4 μM.

Cadaverine scores were most highly associated ($p<0.009$) with odor judge measurement of tongue and saliva, as well as Plaque Index and Gingival Index scores. Significant correlations were also observed between cadaverine and mean BANA scores ($p=0.009$), whole mouth odor judge scores ($p=0.027$) and mean probing depth ($p=0.036$). In contrast, no significant associations were found between cadaverine and VSC levels (Goldberg *et al.*, 1994).

Although cadaverine and putrescine scores were significantly associated with one another ($p<0.001$), putrescine levels, *per se,* were not found to be significantly associated with any of the malodor or dental parameters.

Stepwise forward multiple regression analysis was performed to relate each of the three odor judge measurements to VSC levels, diamine concentrations and BANA hydrolysis (Table 2). In the case of whole mouth odor judge scores, VSC (peak) and BANA factored into the regression equation, yielding a multiple r of 0.568. For tongue odor, VSC (peak) values and cadaverine levels were significantly factored, yielding a multiple r of 0.545.

Table 2

Stepwise Multiple Regression Analysis of Odor Judge Organoleptic Assessments in Terms of VSC, BANA and Diamine Concentrations[a]

Dependent Variable	Intercept	Coefficient for				Multiple r
		Log Peak VSC	Log Steady State VSC	Log [cadaverine]	BANA	
Whole mouth	-6.85	5.82	N.E.[b]	N.E.	0.33	0.568
	0.0157[c]	*0.0008*	*0.6840*	*0.1177*	*0.0104*	*0.0001*
Tongue	-5.43	2.82	N.E.	1.30	N.E.	0.545
	0.0066	*0.0101*	*0.5099*	*0.0023*	*0.1185*	*0.0002*
Saliva	-14.38	N.E.	6.91	2.86	N.E.	0.604
	0.0002	*0.2104*	*0.0015*	*0.0004*	*0.241*	*<0.0001*

[a] Log putrescine was not entered into any of the equations (p= 0.639, 0.435 and 0.189 for whole mouth, tongue and saliva, respectively)

[b] Not entered into the equation

[c] Numbers in italics represent levels of significance

In the case of saliva, VSC (steady state) and cadaverine levels entered the regression equation, with a multiple r of 0.604. Putrescine levels did not enter significantly into any of the regressions.

Outgrowth of Salivary Microbiota in Decarboxylase Medium

Previous investigations related to saliva malodor have often employed putrefied saliva itself. We have found that putrefied saliva yields high levels of volatiles, but does not necessarily smell like bad breath. In an attempt to seek a more representative model, we inoculated saliva into various growth media and tested organoleptically for odor production over time. Satisfactory results were obtained by inoculating early morning saliva 1:25 (v/v) into decarboxylase medium (5 mg/ml Bacto peptone, 3 mg/ml Bacto yeast extract, 1 mg/ml Bacto Dextrose and 20 µg/ml Bacto Bromcresol purple), with optional addition of amino acids to a final concentration of 5 mg/ml.

Table 3
Diamine Concentration, pH, Odor Formation and VSC after 72 Hours Incubation of Saliva in Decarboxylase Medium[a]

Additions	Periodontal disease	Putrescine (μM)	Cadaverine (μM)	pH	Odor judge score[b]	H_2S (ppb)
none	+	398.2	97.3	6.0	3	1140
	-	395.0	55.8	4.5	0	5
lysine (5 mg/ml)	+	39.8	714.3	7.0	3	1460
	–	5.7	19.6	4.5	0	5
ornithine (5 mg/ml)	+	670.0	71.4	7.0	3	1480
	–	489.0	21.5	7.0	1	120
arginine (5 mg/ml)	+	631.4	49.0	8.5	3	200
	–	625.3	72.4	9.0	1	100
histidine (5 mg/ml)	+	295.0	29.0	7.0	2	850
	–	n.d.[c]	n.d.	7.0	1	750
metronidazole (80 μg/ml)	+	32.1	28.4	4.0	0	5
	–	n.d.	n.d.	4.0	0	5
potassium tellurite (10 μg/ml)	+	226.0	28.4	4.0	0	5
	–	n.d.	n.d.	4.0	0	5

[a] Fresh morning saliva was collected from a healthy subject and a subject with moderate periodontitis, and inoculated into decarboxylase medium. Measurements were taken following 72 hours incubation.

[b] Scores were given as follows: 0 - no odor, 1 - slight odor, 2 - moderate odor and 3 - foul odor.

[c] Not determined

To determine whether diamine production is related to malodor elaboration *in vitro*, fresh morning saliva was collected from a subject with no periodontal disease and from a subject with moderate periodontitis. Saliva (0.2 ml) was added to 5 ml of decarboxylase medium alone, or with addition of lysine, arginine, ornithine or histidine, respectively. The final pH was adjusted to

7.0 with HCl. Tubes were incubated standing at 37°C and were checked daily for yellow-to-purple acid-to-base color change. Samples (0.2 ml) were taken at various times for diamine analysis, as described above. The level of volatile sulfides (in peak ppb hydrogen sulfide equivalents) was determined in the headspace of the test tubes themselves, using the portable sulfide monitor, by inserting a plastic 1/4" straw 2 cm into the opened tube.

Results are summarized in Figure 1 and Table 3. In the absence of exogenous amino acids, the cadaverine concentration was about two-fold higher in the sample obtained from the periodontitis subject, following 72 hours incubation, as compared with the sample obtained from the healthy subject. In contrast, putrescine levels were similar (Table 3). When lysine was added to the decarboxylase medium, cadaverine levels increased dramatically over time only in the sample obtained from the subject with periodontal disease (Figure 1), reaching 714.3 µM following 72 hours (Table 3). In contrast, cadaverine levels in the healthy control sample remained stable (Figure 1), reaching only 19.6 µM following 72 hours incubation (Table 3). Addition of other amino acids (ornithine, arginine, histidine) brought about much smaller changes in cadaverine levels (Table 3).

Putrescine levels were generally higher than those found for cadaverine, but were not elevated in the sample obtained from the periodontitis subject (Table 3). As might be expected, addition of both ornithine and arginine resulted in increases in putrescine levels in samples from both subjects. Of interest was the observation that putrescine levels in both samples were strongly inhibited in the presence of lysine. Elaboration of cadaverine and putrescine over time, as well as malodor production, were completely inhibited when 80 µg/ml metronidazole was added (subject with moderate periodontitis). Addition of 100µg/ml potassium tellurite resulted in decreases in cadaverine levels and inhibition of malodor production (subject with moderate periodontitis). Organoleptic malodor scores were higher in all cases for the subject with periodontitis, and VSC levels were also higher, except in the case of arginine. Furthermore, pH levels were much higher in the subject with periodontitis when no amino acid was added and when lysine was present. In most cases, high VSC levels were associated with malodor typical of bad breath. VSC levels and organoleptic ratings were low at pH values below 5.

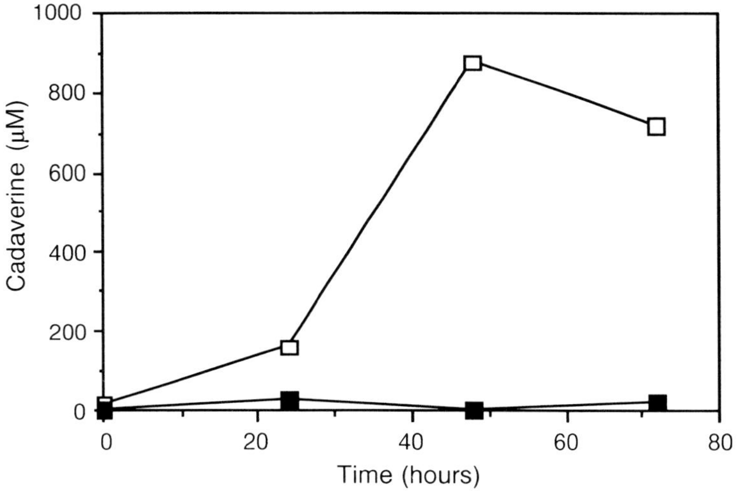

Figure 1. Cadaverine levels in lysine-containing medium as a function of time. Fresh morning saliva from a subject with no periodontal disease (■) and a subject with moderate periodontitis (□) was inoculated into decarboxylase medium containing 0.5% lysine for 72 hours. Cadaverine levels were measured every 24 hours with HPLC as described above.

In vitro Production of Diamines by Periodontal Pocket Inocula

In this experiment, we sampled deep and shallow pockets of 24 patients with periodontal disease. Four sterile paper points were inserted into deep (≥4mm) pockets and were then inoculated together into a test tube containing 5 ml of decarboxylase medium. As control, four paper points were collected from shallow pockets (<4 mm) of each individual and were similarly inoculated. Initial pH of the decarboxylase medium was adjusted to 9.0 in order to increase selectivity. Diamine levels, pH, and organoleptic scores were determined in the test tubes after 0, 24 and 48 hours, as described in the previous experiments.

Results are presented in Table 4. In all cases, mean levels of both cadaverine and putrescine, as well as organoleptic odor scores and mean pH values were significantly higher in test tubes inoculated with paper points from deep, as compared with shallow, pockets.

Table 4
Mean Levels (± SD) of Malodor Production, Diamines, and pH of Incubated Samples Obtained from Deep and Shallow Pockets

Parameter	Time of incubation		
	0 hours	24 hours	48 hours
Malodor production, deep pockets	none	none to weak	moderate to extremely foul
Malodor production, shallow pockets	none	none	none to weak
Cadaverine (μM), deep pockets	53.9 ± 44.1[a]	1,046 ± 1,004[a]	2,278 ± 395[a]
Cadaverine (μM), shallow pockets	23.5 ± 10.8	251 ± 433	1,164 ± 883
Putrescine (μM) deep pockets	59 ± 44b	3,498 ± 2,101[b]	4,906 ± 3,181[b]
Putrescine (μM), shallow pockets	39.5 ± 14.7	1,046 ± 1,374	3,442 ± 2,613
pH, deep pockets	9.0	6.2	6.9
pH, shallow pockets	9.0	5.5	6.2

[a] Significantly higher than corresponding cadaverine levels obtained from shallow pocket samples ($p \leq 0.0005$, paired t-test).

[b] Significantly higher than corresponding putrescine levels obtained from shallow pocket samples ($p < 0.001$, paired t-test).

Deep pocket vs. shallow pocket levels of cadaverine and putrescine for each individual, following 24 hours incubation are shown in Figures 2 and 3, respectively. In 22 of 24 patients, both cadaverine and putrescine levels following 24 hours of incubation were much higher in the deep pocket samples, as compared to shallow pocket samples. After 48 hours incubation, 17 and 21 patients had higher levels of cadaverine and putrescine, respectively, in samples from deep pockets, compared with samples from shallow pockets (not shown). Following 24 hours incubation, Pearson linear regression

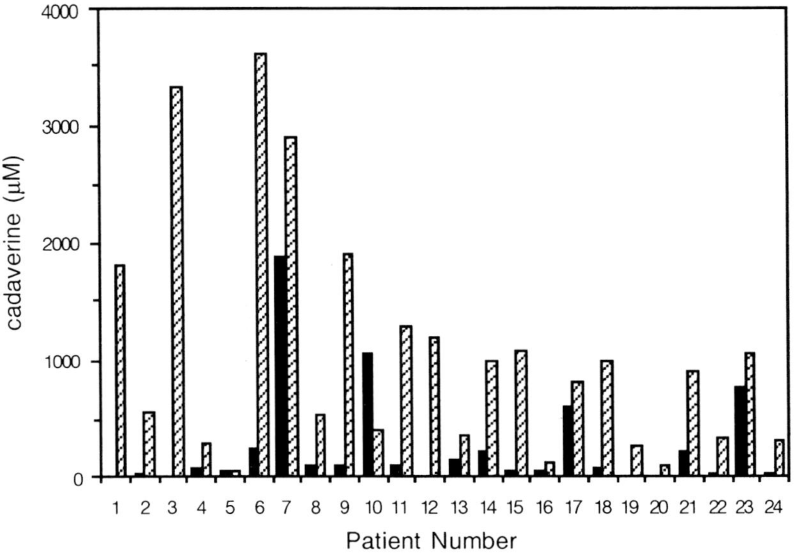

Figure 2. Cadaverine levels of inoculated paper points from pockets of 24 periodontal patients. Four paper points were inserted into deep (▨) or shallow (■) pockets and inoculated in decarboxylase medium. Cadaverine levels (μM) were measured by HPLC following 24 hours incubation.

analysis showed that putrescine and cadaverine levels in samples from shallow pockets were significantly associated with one another ($r=0.67$ $p<0.001$); the corresponding correlation between putrescine and cadaverine in deep pocket samples was only 0.27 ($p>0.05$). Significant correlations following 48 hours incubation were not observed.

Discussion

The experiments shown here, taken together, suggest that the levels of diamines (cadaverine in particular) in saliva, as well as media inoculated with samples obtained from periodontal pockets, are associated with production of oral malodor. The results *per se* do now show that either or both diamines are actual components of oral malodor. Nevertheless, these

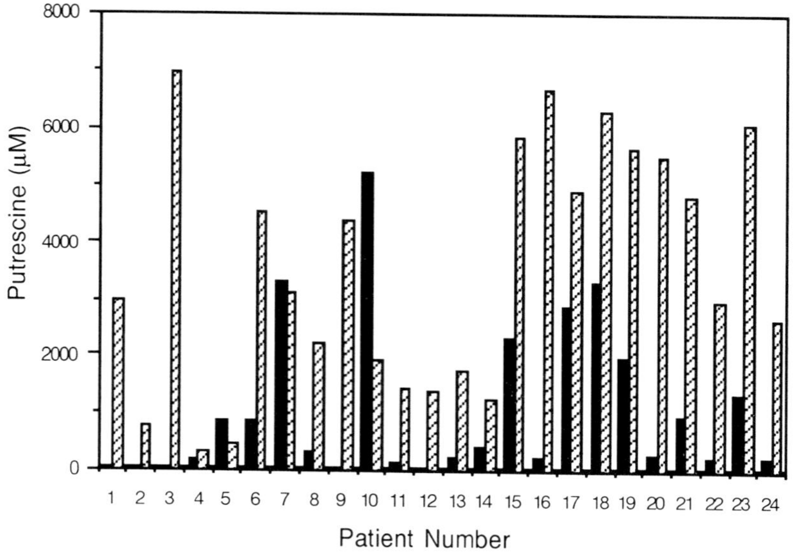

Figure 3. Putrescine levels of inoculated paper points from pockets of 24 periodontal patients. Four paper points were inserted into deep (▨) or shallow (■) pockets and inoculated in decarboxylase medium. Putrescine levels (µM) were measured by HPLC following 24 hours incubation.

compounds are malodorous molecules which are produced as the result of bacterial proteolysis, and which can be volatilized upon drying from human skin (Kleinberg and Codipilly, this volume).

Several differences were observed between putrescine and cadaverine elaboration in the three approaches described here. In the clinical study of 52 subjects, salivary cadaverine levels correlated with malodor- and periodontal disease-associated parameters, whereas putrescine levels did not. Furthermore, as described by Goldberg *et al.* (1994), multiple regression analysis of dental indices (PI and GI) yielded highly significant positive associations with cadaverine levels, combined with significant *negative* associations with putrescine concentrations.

In the second experimental approach described in the present chapter, saliva from a periodontitis subject was compared with saliva from a healthy subject.

Following inoculation into decarboxylase medium, the periodontitis-associated sample showed higher cadaverine levels which were dramatically increased upon addition of lysine, concomitant with odor production. In contrast, the control sample yielded low cadaverine levels which did not respond to exogenous lysine addition. In addition, putrescine levels in samples inoculated with saliva of the two subjects were much more similar to one another. In several instances, relatively high putrescine levels did not lead to detectable odor. Two agents which inhibit growth of Gram-negative microbiota (metronidazole and potassium tellurite) completely inhibited odor production, VSC levels, and brought about reductions in pH and diamines. Of considerable interest was the dramatic inhibitory effect of lysine on putrescine elaboration in the periodontitis-associated and no periodontitis-associated samples (90 and 98% inhibition, respectively).

In the third experimental approach, we have shown that both diamines are elaborated during outgrowth of samples obtained from periodontal pockets. In both cases, deep pocket samples yielded higher diamine levels than corresponding shallow pocket samples. Putrescine and cadaverine levels were significantly associated with one another when comparing samples from shallow pockets, but not deep pockets.

In all three experiments, cadaverine levels were associated with malodor elaboration and periodontal health. The role, if any, of putrescine, remains complex. Putrescine may be produced by several metabolic pathways (*e.g.*, decarboxylation of ornithine, deamination of arginine). Furthermore, data presented here suggest that putrescine synthesis is greatly inhibited by lysine decarboxylation. Further studies are required to (i) explore the apparent discrepancy between the *in vivo* and *in vitro* results regarding the relationship between putrescine and oral malodor; (ii) demonstrate the presence of cadaverine and/or putrescine in the headspace itself; and (iii) identify the oral microbiota which produce these compounds in health and disease.

Acknowledgments

We thank S.A. Berger and R. Lamed for their advice, and Y. Mazor for excellent technical assistance.

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6

Oral Malodor and Periodontal Disease

Ken Yaegaki

Periodontal pathogenesis of volatile sulfur compounds (VSC) is described. VSC increase the permeability of oral mucosa, collagen solubility, protein and collagen degradations, and reduce protein, collagen and DNA synthesis.

Biochemical features of oral malodor caused by periodontal disease were shown as follows: The amounts of VSC and methyl mercaptan/hydrogen sulfide ratio in mouth air from patients with periodontal involvement were eight times greater than those of control subjects. It was demonstrated that in periodontal patients: (i) 60% of the VSC was produced from the tongue surface; (ii) the amount of tongue coating was four times greater than in control subjects; and (iii) VSC production and the methyl mercaptan/hydrogen sulfide ratio of the tongue coating were increased markedly. 2-Ketobutyrate, a by-product of methionine metabolism to methyl mercaptan, increased in the saliva of patients with periodontal disease, suggesting that methionine metabolism to methyl mercaptan increases in the oral cavity of patients with periodontal disease. As free methionine is the main source of methyl mercaptan production, the methionine supply from the gingival fluid into the oral cavity of periodontal patients was estimated.

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The results indicated that the ratio of methionine to whole free amino acids was significantly higher than that of cysteine. It has been suggested that not only periodontal pathogenic microorganisms, but also the tongue coating and gingival fluid are factors which enhance VSC production in periodontal patients.

Introduction

In 1964 Professor Tonzetich from the University of British Columbia, Vancouver, Canada, (the pioneer of modern bad breath research), started the biochemical study of oral malodor. He produced a number of excellent works on oral malodor research and has clarified the etiology. One of his more notable achievements was the discovery that volatile sulfur compounds (VSC) are the principal components of oral malodor (Tonzetich and Richter, 1964; Tonzetich, 1971), whereas other odorous molecules produced by the oral microorganisms only modify the quality and intensity of oral malodor (Tonzetich, 1971; Tonzetich and Ng, 1976). In this chapter, the intensity of oral malodor is expressed as a concentration (ng/10 ml mouth air) of VSC, such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), dimethyl sulfide (CH_3SCH_3) and total sulfur.

From ancient time (Eourdet, 1771; Geist, 1956) it was known that periodontal disease frequently causes halitosis. Hippocrates described a remedy for oral malodor (Geist, 1956) caused by periodontal disease and said "If the gingiva become healthy again, the offensive odor vanishes". Periodontal disease frequently involves oral malodor with relatively higher intensity compared with subjects without periodontal disease, Tonzetich (1971) estimated VSC concentrations in mouth air with gas chromatography. He found that VSC concentrations increased with the severity of periodontal disease (1978). Other studies have also indicated that VSC production increases with periodontal disease (Rizzo, 1967; Nara, 1977; Fukushima, 1986; Yaegaki and Sanada, 1992a,b). Bacteriologic studies (Tachibana, 1957; Tonzetich and McBride, 1981) suggest that an increase of VSC may, in part, be related to the activity of VSC-producing microorganisms, whose populations are higher in periodontal pockets than in normal gingival sulcus. However, because offensive malodor frequently occurs in an orally healthy population, and as average concentrations of VSC in orally healthy subjects are higher than the threshold of the human smell (Yaegaki and Sanada, 1992a,b), not only periodontal factors but also other oral environmental factors may affect VSC production in the oral cavity. If this is true, the property of malodor in the orally healthy population may be different from that in periodontal patients. In this chapter, the relationship of VSC, oral environmental factors and periodontal status are discussed.

On the other hand, Tonzetich and his group concluded that VSC, which are themselves very toxic, may have an important role in the etiology of periodontal disease and may accelerate periodontal disease. VSC increase collagen solubility (Johnson and Tonzetich, 1979) and the permeability of the oral mucosa, which is very similar to the epithelium of periodontal pockets (Ng and Tonzetich, 1984), and decrease the protein and collagen synthesis (Johnson and Tonzetich, 1982). Therefore, it is important to comprehend the influence of VSC on the periodontal tissues in order to conduct research into pathogenesis of periodontal disease. The periodontal pathogenesis of VSC is also discussed based on the results obtained by Tonzetich and his group.

The Effect of VSC on the Permeability of Oral Mucosa

Ng and Tonzetich (1984) report the effect of H_2S and methyl mercaptan on the permeability of the non-keratinized oral mucosa. The gingival sulcular epithelia are considered to be barriers to the penetration of microbial substances from the gingival sulcus or periodontal pocket. It has been suggested that the onset of periodontal disease follows the initial damage to the epithelial barrier (Page and Schroeder, 1976; Gaffar *et al.*, 1981). The bacterial products induce a change in the barrier structure to permit an increase in accessibility of microbial toxic substances, such as endotoxin, to the underlying connective tissues. The basal membrane and the extracellular components in the epithelium, such as proteoglycan, can collectively act as the tissue barrier against microorganisms and their products.

Ng and Tonzetich (1984) report that the comparison of initial and final permeability coefficient values showed that permeability was not affected by exposure of porcine sublingual non-keratinized mucosa (as a model for the crevicular epithelia) to control air, but was markedly increased by the presence of VSC (Table 1). The magnitude of the percentage increase in permeability was dependent on both the concentration and the duration of VSC application. Ng and Tonzetich (1985) also found that endotoxin and prostaglandin E_2 penetrations into the mucosa increase significantly. They

Table 1
Percentage Increase in Permeability of Oral Mucosa
Subjected to Various Time Periods to H_2S or CH_3SH[a]

	% Increase in permeability		
Exposure time (min)	CO_2/air[b]	H_2S	CH_3SH
5	-	10.6	-
30	-	23.3	19.0
60	-6.9	59.1	34.6
120	-6.9	-	103.0
180	9.3	61.7	73.0

[a] 15.0 ng H_2S or CH_3SH per ml 95% air/5% CO_2
[b] 95% air/5% CO_2 control atmosphere

observed that H_2S retained by the epithelial tissues and thiol-tissue complex are fairly stable and resist elution by phosphate buffered saline (PBS). Thus, Ng and Tonzetich (1984) suggest that VSC react with the basal membrane and/or intercellular matrices and in the process alter the permeability. The efficiency of the epithelial barrier may be impaired due to localized breaks in the basal membrane (Muller-Glauser and Schroeder, 1982), but Ng and Tonzetich (1984) also suggest that VSC induce de-aggregation of proteoglycans, which are extracellular matrices of connective tissues, by cleaving disulfide bonds which may cause an increase in permeability. They demonstrated that treatment of mucosa with 0.22% zinc chloride nullified the effect of VSC and restored the permeability to a state similar to that observed in control. As the use of 0.2% chlorhexidine gluconate mouthwash, which is considered to be very effective, is forbidden by the Japanese Ministry of Health and Welfare (it causes very severe anaphylactia in our population), I have not compared it to zinc chloride mouthwash. But I do consider zinc mouthwash, which reduces VSC concentration by more than 90% for more than 3 h, to be one of the most effective deodorants for oral malodor in orally healthy populations (Tonzetich, 1978; Yaegaki and Suetaka, 1989a; c). These results support the fact that zinc is frequently used in dermatology to stabilize tissue proteins in wounds or in dermatological dieseases.

Collagen Metabolism by Gingival Fibroblasts in the Presence of VSC

Tonzetich *et al.* (1986) describe the effect of methyl mercaptan on collagen metabolism in human gingival fibroblast cultures. The collagen content is the net difference between its rates of synthesis and degradation. Since methyl mercaptan causes a marked reduction in collagen content of fibroblast culture, collagen production rate and degradation rate were estimated (Tonzetich and Yaegaki, 1986). Human gingival fibroblast culture was pulsed 30 min and 12 h with [^{14}C]-proline in the presence of 10 ng methyl mercaptan/ml control air. The amount of collagen was determined by assaying for hydroxyproline using the method of Yaegaki *et al.* (1986). Methyl mercaptan was found to decrease synthesis and increase extracellular degradation of collagen. The 30 min pulse study indicates about 40% suppression of synthesis, and a 2-fold increase of intracellular degradation of collagen was observed in both 30 min and 12 h pulsed cultures. Extracellular degradation of collagen in 12 h pulsed cultures also increased by 13-fold. The author implies that since thiol decreases stability of the biomembrane, the acidic hydrolytic enzymes, such as proteinases, originating from lysosomes, may increase in the presence of methyl mercaptan. Thus, VSC may induce a collagen loss in periodontal tissue with severe inflammation.

Effect of VSC on Wound Healing

Yaegaki (1986) reported that VSC delay wound healing. Punched-out wounds were made on the rat skin, then sealed with a Teflon-coated metal cap with a sealed injection hole of 1 mm in diameter. Once daily, 100 mg of methyl mercaptan was dropped into the cap through the injection hole. One week later, lesions were biopsied and the specific stain for collagen was employed. Figure 1 shows the control; many mature collagen fibers are observed and the wound is completely covered with epithelia. In the test group, the amount of collagen fibers is less than that in control (Figure 2). No formation of epithelia is observed on the wound, but an ulcer lesion still remains. According to PAP stain for type IV collagen in the basal membrane, no collagen (type IV) and no basal membrane are observed in the test group (data not shown), although the membrane is established in the control lesion. Thus, it was concluded that VSC are very toxic in the process of the wound healing after oral surgery, *i.e.*, periodontal or dental implant surgery. It is suggested that zinc mouthwash counteracts the toxicity of VSC.

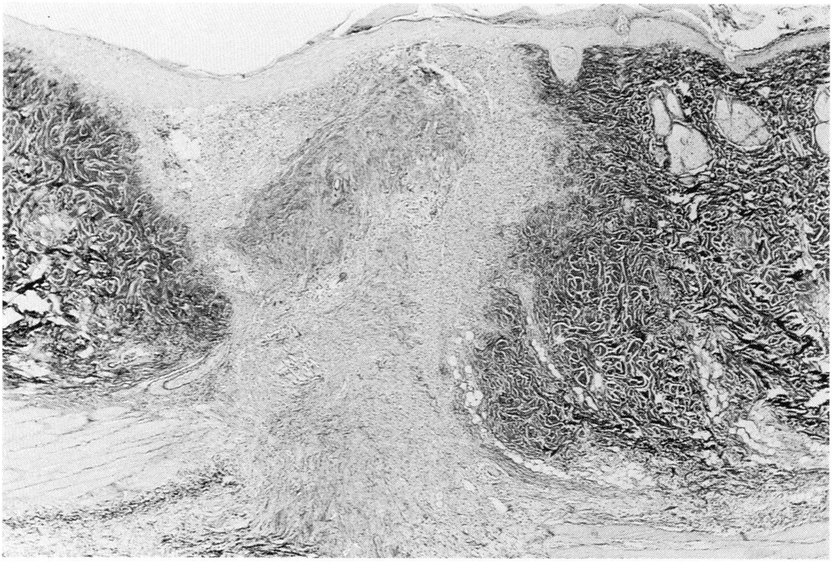

Figure 1. The wound is filled with collagen fibers; formation of epithelium is observed (control).

Effect of VSC on the Active Oxygen Production in Human Leukocytes

Active oxygen has been considered to be very toxic to human gingival tissues. Since many leukocytes migrate into the periodontal pocket (Dreizen *et al.*, 1956; Nara, 1977; Raeste and Aura, 1978), it has been considered that active oxygen is produced in the periodontal pockets.

Dr. Kato, Department of Oral Microbiology, The Nippon Dental University School of Dentistry at Niigata, and I have determined the effect of active oxygen production in human polymorphonuclear leukocytes (PMN) using chemiluminescence (unpublished data). In FMLP (*N*-formyl-*l*-methionyl-*l*-leucoyl-*l*-phenylalanine) stimulation, production of active oxygen tends to decrease in the presence of methyl mercaptan; however, the production of active oxygen in *Fusobaterium* stimulation was dramatically increased by methyl mercaptan (Table 2). These results suggest that the production of active oxygen in the periodontal pocket is stimulated by VSC originating from oral microorganisms, and that an elevated amount of active oxygen would accelerate the destruction of periodontal tissues.

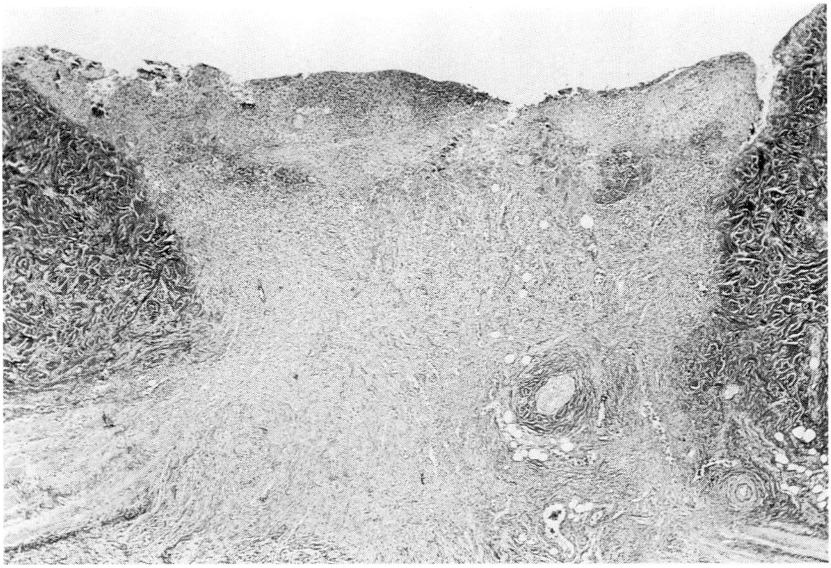

Figure 2. The amount of collagen fibers is less than that in control. No formation of epithelium is observed (test: exposure to methyl mercaptan).

Summary of Periodontal Pathogenesis of VSC

Table 3 shows the summary of periodontal pathogenesis of VSC. Since the toxicity of VSC is equivalent to that of cyanide (Skrede, 1969), VSC should be considered as one of the most important periodontal toxins similar to other products of microorganisms, such as endotoxin. Furthermore, it is expected that zinc would be widely used for the purpose of periodontal therapy.

Characteristics of Halitosis and Periodontal Evaluations

Higher concentrations of VSC are observed in mouth air from patients with periodontal disease (Rizzo, 1967; Nara, 1977; Tonzetich, 1978; Yaegaki and Sanada, 1992a,b). Tonzetich (1978) has shown that VSC concentration in mouth air increases with the total pocket depth; Tonzetich and McBride (1981) demonstrate that a pathogenic strain of *Porphyromonas gingivalis* produces much more methyl mercaptan than H_2S. Yaegaki and Sanada (1992a,b) found that the VSC concentration is higher in patients with probing

Table 2
Effect of Methyl Mercaptan on Chemiluminescence of Human PMN

Stimulation	Change (%) of Chemiluminescence[a]
FMLP	- 36.9 ± 13.8 (N = 6)
Fusobacterium	+ 48.9 ± 33.5 (N = 6)

[a] Methyl mercaptan (10 ng/10 ml air) flowed during 30 min. Peak value was estimated as volume of chemiluminescence (Kato and Yaegaki, 1993 unpublished data).

depths of 4 mm or more than in subjects with probing depths of less than 4 mm. In particular, the methyl mercaptan concentration is significantly higher than in patients with periodontal disease than in controls ($p<0.01$; Figure 3).

The bleeding index, which is derived from the number of bleeding points caused by probings, was used to compare the extent or severity of periodontitis (Mikami *et al.*, 1987). Total VSC (ng/10 ml mouth air) increased with bleeding index (Figure 4). The production of H_2S in periodontal pockets was demonstrated by inserting leaded filter papers into the pockets; however, this method is not quantitative (Rizzo, 1967).

Thus, it is evident that periodontal disease causes high concentrations of VSC in mouth air, with consequent quantitative changes in bad breath. However, it remained to be determined whether the composition of VSC associated with oral malodor caused by periodontal disease was the same as that of orally healthy subjects. Yaegaki and Sanada (1992a,b) determined the methyl mercaptan/H_2S ratio using the gas chromatography method. These two compounds, rather than dimethyl sulfide, are considered to be the main components of VSC in mouth air (Tonzetich and Kestenbaum, 1969; Tonzetich, 1971; Tonzetich, 1978). It was found that the methyl mercaptan/H_2S ratio in patients with probing depths of 4 mm or more (Figure 3) was much higher than that of controls, and that it increased in proportion to the bleeding index (Figure 4), which reflects the extent of periodontal disease (Yaegaki and Sanada, 1992a,b).

Table 3
Periodontal Pathogenesis of VSC

1. Increase of permeability of oral mucosa
2. Increase of penetration of PGE_2 and endotoxin into oral mucosa
3. Increase of solubility of collagen
4. Increase of collagen degradation
5. Suppression of DNA synthesis
6. Suppression of proline transport into cytosol
7. Increase of active oxygen production by PMN
8. Suppression of protein synthesis

The maximum probing depth was determined and compared with the methyl mercaptan/H_2S ratio in each patient with periodontal disease. Figure 5 shows that the ratio increases with probing depth (Yaegaki and Sanada, 1992a,b). The group with probing depths of 3 mm or less had a ratio of 0.37; with 4 mm probing depths the ratio was 0.96, it was 3.49 with 5 mm depths and 6.1 with depths in excess of 5 mm. Therefore, these results indicate that methyl mercaptan is the main component of VSC in patients with periodontal involvement. On the other hand, H_2S is the main constituent in an orally healthy population. The methyl mercaptan/H_2S ratio increases with the severity of periodontal disease.

Since the etiology of oral malodor in orally healthy subjects may differ from that in the periodontal patients, a criterion to estimate or investigate the oral malodor in orally healthy subjects might distinguish it from that in the periodontal disease.

Methionine Metabolism in the Oral Cavity With Periodontal Disease

Methyl mercaptan concentration in mouth air from periodontal patients increases compared with orally healthy subjects. H_2S is produced from thiols (Tonzetich and Catherall, 1976; Harada *et al.*, 1989), such as cysteine, and methyl mercaptan is produced from methionine. On the other hand, it was shown that H_2S can be converted to methyl mercaptan in the oral cavity

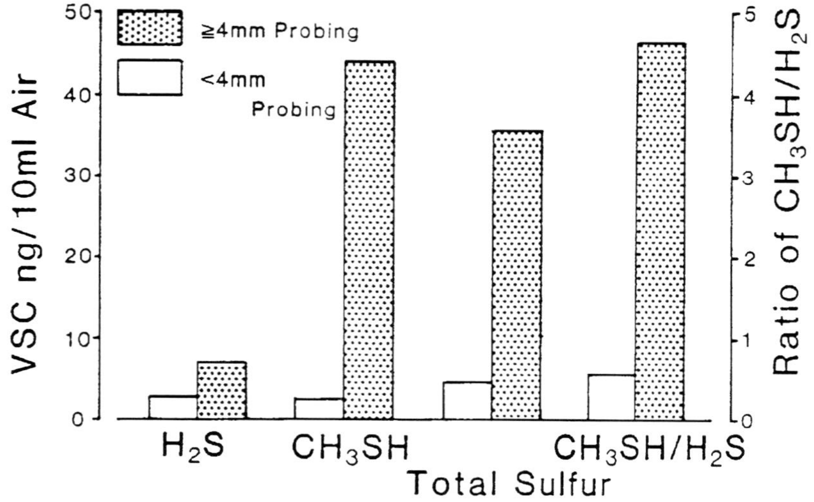

Figure 3. VSC production in mouth air from periodontal patients (N=17) with gingival probing depth of 4 mm or more. In comparison with control (N=14) with a probing depth of less than 4 mm, a large amount of VSC production was observed, and the methyl mercaptan/H_2S ratio was markedly increased.

(Kaizu *et al.*, 1978). Thus, it is not clear whether the elevated methyl mercaptan concentration in periodontal patients originates from elevated H_2S production or methionine metabolism.

2-Ketobutyrate is a by-product of methionine metabolism which produces methyl mercaptan. 2-Ketobutyrate concentration in saliva from subjects with periodontal disease was determined by the HPLC method (Yaegaki and Sanada, 1992a,b). The results demonstrate that 2-ketobutyrate increased by more than 6-fold in the mixed saliva from periodontal patients compared with orally healthy subjects (Table 4). These observations strongly support the fact that the production of methyl mercaptan from methionine is accelerated in periodontal patients. It has been shown that the elevated concentration of methyl mercaptan does not originate from the conversion of H_2S to methyl mercaptan.

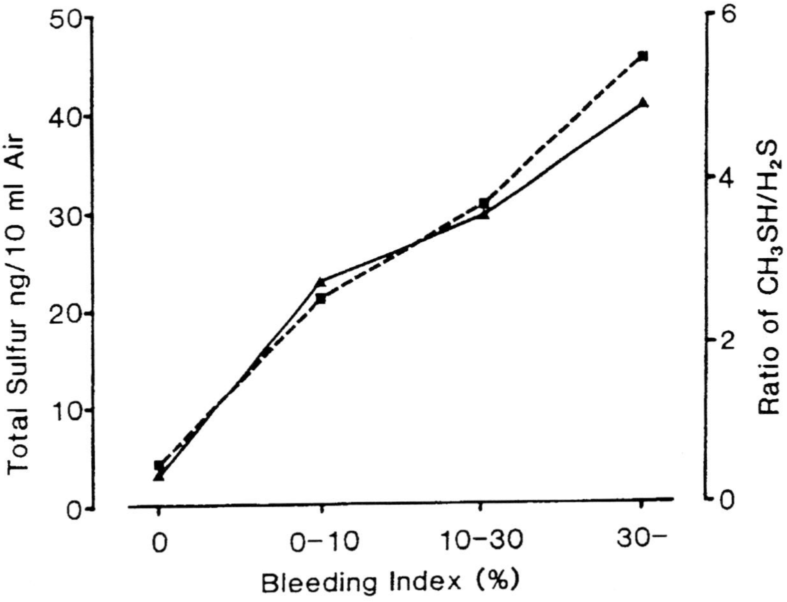

Figure 4. VSC production and methyl mercaptan/H_2S ratio increased with bleeding index. 0 - N=11; 0-10 - N=7; 10-30 - N=4; >30 - N=9. Broken line: the ratio; solid line: total VSC.

Methionine in Gingival Fluid

Tonzetich and McBride (1981) have shown that periodontal pathogenic microorganisms produce 12 times as much methyl mercaptan as H_2S. The nature of the microorganisms is an important reason why the production of methyl mercaptan is elevated in periodontal patients. There is, however, another plausible explanation.

The VSC concentrations in mouth air at 2 min after mouth rinsing with amino acid or protein solutions, such as L-cysteine, L-methionine and casein, have been determined (Shibuya *et al.*, 1983; Ishikawa *et al.*, 1984). It has been demonstrated that only free methionine is a good source of methyl mercaptan production. Therefore, the free methionine supply in the oral cavity with periodontal disease was investigated.

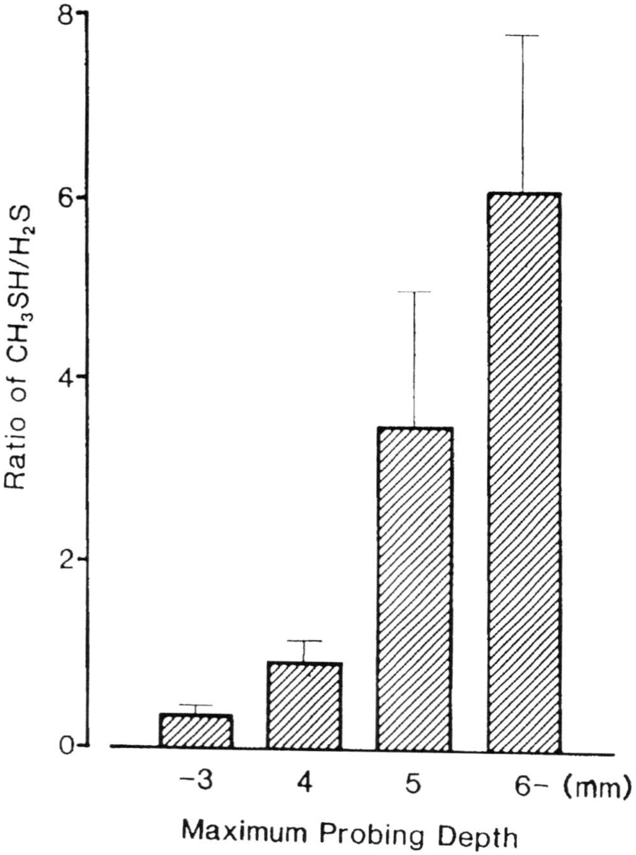

Figure 5. Ratio of methyl mercaptan/H_2S increases with maximum probing depth in each subject. 0-3 mm (N=9); 4 mm (N=6); 5 mm (N=8); 6 mm (N=11).

There is no difference between methionine concentration in the saliva samples from the patients with periodontal disease and controls. But, the ratio of methionine to whole free amino acids in gingival fluid from periodontal pockets is significantly higher than those of cysteine and cystine (Yaegaki and Sanada, 1992a,b). This implies that gingival fluid of subjects with periodontal disease is a good source for methyl mercaptan rather than H_2S production. The taurine ratio was 21.4%, which is markedly high in

Table 4
2-Ketobutyrate in Saliva from Periodontal Patients

	Patient group	Control group
2-Ketobutyrate nm/ml saliva	32.7 ± 4.1 (N=38)	0.6 ± 0.5 (N=16)
Average (mm) of total pocket depth	202.7 ± 23.6	–

comparison with others; the metabolism of sulfur-containing amino acids in the gingival tissues and/or high concentration of taurine originating from serum may elevate the concentation in the gingival fluid.

It has been concluded that the periodontal pocket possesses an appropriate environment for methyl mercaptan production, such as periodontal pathogenic microorganisms and an adequate supply of free amino acids. The observation that very high concentrations of dimethyl sulfide to which methyl mercaptan is dimerized, occur in periodontal pockets supports the finding that methionine metabolism is accelerated in periodontal pockets.

Sulfur Source in Salivary Cellular Elements

Saliva is one of the main sources of sulfur converted to oral malodor. Since filtration removes most of the organic sulfur sources from saliva, the salivary cellular elements are a principle source of the thiol and disulfide groups used to produce H_2S (Tonzetich and Johnson, 1977).

The distribution of thiols and disulfides in salivary cellular elements was analyzed with the Percoll density gradient centrifugation technique (Yaegaki and Suetaka, 1989d). Disulfide is distributed mainly in the intact cells, whereas the thiol concentration is lower in the intact than in the damaged cells. The intact squamous cells are distributed in the upper layer fraction (density $\leq$ 1.051). The recovered cells in this fraction were 40-50% in protein content. The degraded cells, which were out of shape or lost nucleus, distributed in the middle density (1.051-1.076) and lower layer fraction (density ≥ 1.076). The density of the cell was also found to increase in the process of the cell degradation. The 24 h incubation study of saliva showed that the intact cells decreased markedly, and the degraded cells increased in the middle layer fraction. Thiol concentration (nm/mg protein) in the lower layer fraction was double of that in the upper layer fraction. Yaegaki and Suetaka (1989a,b) describe that cell destruction increases the specific gravity of cells and thiol concentration but decreases disulfide content. Yaegaki and Suetaka (1989a,b) suggest that high density cells might accumulate on the tongue dorsum for a long time and be the major source of VSC precursors.

Sulfur Source in Leukocytes Migrating into the Oral Cavity

Leukocytes possess large quantities of sulfur-containing amino acids (Barrnet, 1953), which could be utilized for oral malodor production. Leukocytes migrate from periodontal pockets; the number in saliva increases in patients with periodontal disease (Dreizen *et al.,* 1956; Nara, 1977; Raeste and Aura, 1978). Furthermore, the number of leukocytes in saliva correlate with some periodontal evaluations and methyl mercaptan concentration (Nara,1977). It has been suggested that leukocytes are a major source of VSC in periodontal patients.

The salivary thiol concentration does not increase significantly in the saliva of periodontal patients, since thiol is too reactive to exist in its free form. However, Yaegaki and Suetaka (1989b) describe that the disulfide content (Figure 6) and concentration in saliva correlate with the severity of periodontal disease. The disulfide content and concentration also correlate with the periodontal pocket distribution and the mean pocket depth. Therefore, they conclude that the content of sulfur-rich proteins or sulfur-containing amino acids, which originate from the migrated leukocytes from periodontal pockets, increase in saliva from patients with periodontal disease.

Other Blood Components Stimulate VSC Production

Yaegaki and Sanada (1992a) demonstrated that VSC concentrations and the methyl mercaptan/H_2S ratio increases in mouth air in proportion to the bleeding index. As the bleeding index and concentration of erythrocytes in saliva are mutually related (Yasui *et al.*, 1985), other blood components, as well as leukocytes originating from periodontal pockets may accelerate

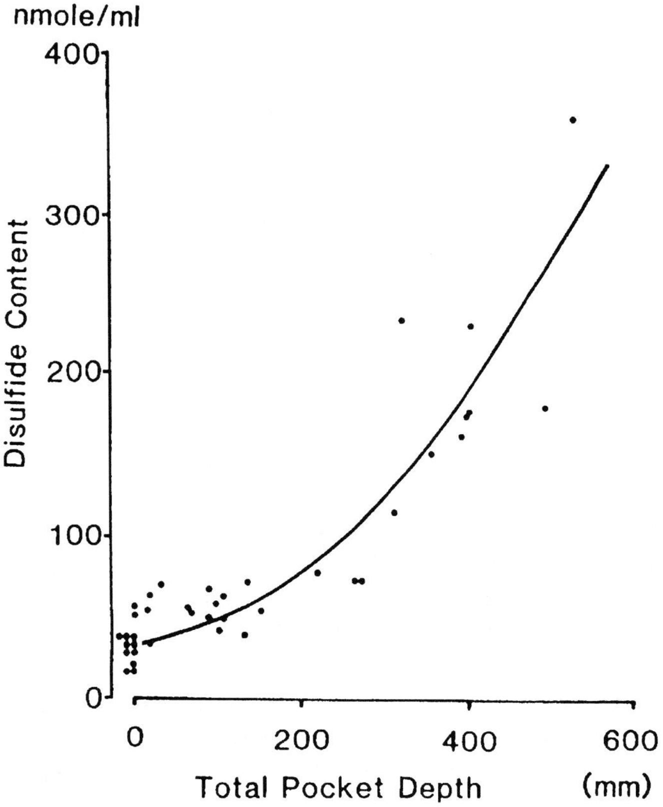

Figure 6. Disulfide concentration in saliva increased with total pocket depth in each subject. $Y = 40.5275 + 0.0299X + 0.0009X^2$ with correlation coefficient determination of 0.8407 ($p<0.001$).

VSC production. Since *Porphyromonas gingivalis* requires hemin, which originates from hemoglobin for growth, and as vitamin K, which exists in the blood at high concentrations also stimulate their growth, the growth rate increases in proportion to the hemin concentration. Therefore, it has been concluded that blood components, which increase in the oral cavity with periodontal disease, enhance the bacterial activities which produce VSC.

VSC Production on Tongue Coating With Periodontal Disease

Tongue coating is generally believed to be one of the main sites of VSC production in orally healthy subjects whose oral malodor is caused mainly by H_2S (Kaizu, 1976; Tonzetich, 1978; Kojima, 1985). Tongue coating is comprised of desquamated epithelial cells, blood cells and bacteria. More than 100 bacteria may be attached to a single epithelial cell on the tongue dorsum, whereas only some 25 bacteria are attached to each cell in other areas of the oral cavity (Iwata *et al.*, 1985). Therefore, it has been considered that tongue coating has the putrefactive activity to produce a large amount of VSC from these sulfur sources. A preliminary study has suggested that the volume of tongue coating tends to increase in cases of periodontal involvement (Abo, 1943). Therefore, I have been investigating whether tongue coating contributes to VSC production in patients with periodontal disease.

To determine the effect of cleaning the tongue surface on VSC production, tongue coating was removed as follows: saliva on the dorsal surface was removed with air flush and pure pulp tissue paper (Kimwipe). The tongue coating was removed with a tongue scraper of a small spoon type developed by the author, from the terminal sulcus of the tongue to the apex. The tongue dorsal surface was then wiped with cotton pellets immersed in physiologic saline. Immediately after cleaning, VSC concentrations in mouth air were determined by gas chromatography, which was equipped with a special device for sample injection. Table 5 shows that VSC decreased by 51.8% in the control, and by 49.0% in the periodontal patients. The methyl mercaptan/H_2S ratio decreased by 55.5% in the control group and 34.8% in the periodontal patients (Yaegaki and Sanada, 1992a,b). These results indicate that the tongue dorsal surface is one of the main sites of VSC production in orally healthy and periodontal involvements, but produces mainly H_2S in both groups.

The amount of tongue coating and VSC production were determined (Table 6). The periodontitis patient group had much more tongue coating than in the controls, and VSC production from the coating in the periodontitis patient group was estimated to be more than four times that of the control group. The methyl mercaptan/H_2S ratio was also much higher than in controls (Yaegaki and Sanada, 1992a,b).

Table 5
Effect of Removal of Tongue Coating on VSC Production

Removal of	Total Sulfur (ng/10 ml)			CH_3SH/H_2S
tongue coating	< 4 mm	≥ 4 mm	< 4 mm	≥ 4 mm
Before	8.3 ± 4.0	36.5 ± 12.0	0.9 ± 0.1	4.5 ± 1.1
After	4.0 ± 1.6	18.6 ± 6.8	0.4 ± 0.2	3.0 ± 14.6
% Reduction	51.8 ± 12.8	49.0 ± 16.4	55.6 ± 12.3	34.8 ± 14.6

It is suggested that tongue coating as well as periodontal pockets are main sources of VSC production in periodontal diseased patients and play an important role in accelerating VSC production. Yaegaki and Sanada (1992a) conclude that much more VSC is produced on the tongue dorsal surface and removal of the tongue coating does reduce VSC in orally healthy subjects and in periodontal patients.

Other Topics: Bad Breath Detector Made in Japan

Recently, some portable bad breath detectors have been introduced into clinical fields by Japanese companies. These detectors are equipped with the same kind of semiconductor gas sensors (Tsunoda, 1988). My study (unpublished data) shows that many misjudgments by Japanese semiconductor gas sensors were found when comparing their data with those from gas chromatographic procedures established by Tonzetich (1971). The sensor cannot discriminate oral malodor from other organic vapors or gases, and it is considered that the specificity of the sensor to VSC may be less than that of the Halimeter (Interscan Co, Chatsworth, CA). The sensor sometimes causes failure in the treatment of patients with psychosomatic factors, because those who know that the semiconductor is unreliable, cannot trust the doctor who uses the data from the sensor. Many patients had already purchased a compact and personal type of bad breath detector, which is equipped with the same kind of sensor as the former portable detectors, before visiting my office.

Table 6
VSC Production From the Tongue Coating and Amount of Tongue Coating

Probing depth	Wet weight (mg)	VSC (ng/10 ml)	CH_3SH/H_2S ratio
< 4 mm (N=14)	14.6 ± 7.5	4.3 ± 3.1	1.0 ± 0.3
≥ 4 mm (N=17)	90.1 ± 11.0	18.6 ± 6.8	31.3 ± 18.1

Treatment must begin by gaining the confidence of the patient who is psychosomatic. Thus, the semiconductor sensors cannot be recommended for use in bad breath examination.

At present, as gas chromatography is not suitable for a dental office because of the cost and the size, we have to use a portable detector on our patients. Therefore, the development of a more reliable portable detector for oral malodor is required.

Recently, Ehara (1993) has reported on the odor visualization method, which is a radar chart plotting, using six metal oxide semiconductors. The method discriminates good smells, which are indicated by a spear-shape on the chart, from bad smells. This method is expected to be introduced into oral malodor research and clinics.

Other Topics: Psychosomatic Halitosis in Japan

In Japanese dentistry, psychosomatic halitosis is diagnosed as so-called "self-halitosis" (Yaegaki *et al.,* 1993). Self-halitosis has been defined as "motivations which lead the patient to have too much interest and become extraordinarily conscious of their own bad breath. However, the malodor cannot be perceived physically, medically and/or objectively" (Uchida, 1974). I have occasionally detected a sufficient amount of VSC in mouth air from patients who were diagnosed with "self-halitosis" by specialists, using gas chromatography or the Halimeter, but cannot propose a definition of this disease (Yaegaki *et al.,* 1993). For psychosomatic halitosis, "halitophobia" (Rosenberg and Leib, this volume), would be more accurate than "self-halitosis". I have found a minute number of real "self-halitosis" patients without bad breath. The VSC concentrations in those patients are very low all day; periodontal therapy, tongue cleaning and zinc mouthwash are not effective. Oral hygiene and salivary flow rate are fairly good. However, I believe that the disease may fall under the category of psychiatry. In my experience, anthrophobia, shizophrenia, paranoia and depressive psychosis have been found. A case of depressive psychosis follows.

A 60-year-old male university professor was referred to the author by a periodontal specialist on October 22, 1993. Early in the morning, the VSC concentration was determined to be 45 ppb by the Halimeter. The patient was instructed to abstain from oral hygiene including oral rinsing and ingesting food and liquid on the morning of the examination. Since the periodontal therapy had been completed by a specialist, and as tongue coating was found, tongue cleaning with a tongue brush was instructed and zinc mouthwash was prescribed. Two weeks later, he returned and complained that offensive odor still remained. Therefore, he was referred for psychosomatic counseling by the author. He was found to be very depressed since his wife's death, a year prior, and was exhausted by the politics among the professors at his school. A long traveling vacation was recommended, but he could not accept it. He was afraid that if he took a long vacation, he would have a bad reputation. The patient was, therefore, referred to a specialist of psychiatry. Unfortunately, the patient committed suicide four months later on March 23, 1994. Clinicians who are concerned with halitosis patients must search for the dangerous signals behind bad breath.

Acknowledgments

The author is very grateful to Professor T. Kitamura, Chairman, Department of Preventive Dentistry, School of Dentistry, Tsurumi University, Yokohama Japan, for his valuable support, excellent suggestions and advice to the author.

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7

Relationship of Oral Malodor and Periodontitis

Christopher A.G. McCulloch* and Anne Bosy

Kleinberg and Westbay (1990) have indicated that oral malodor may be related to microbial metabolism, specifically the putrefaction of debris by oral bacteria. Therefore the measurement of oral malodor could be used as an indicator of diseases in which bacterial metabolism is central to pathogenesis. Historically, the primary interest in oral malodor research has focused on social interaction. Are we going to offend our neighbors if we have this condition? As early as the time of Hippocrates, physicians have discussed diagnosis and treatment of oral malodor. Some 90 years ago, the great Canadian physician, Sir William Osler, indicated that the detection of oral malodor can be a good indicator of oral and certain systemic diseases. Our specific interest is the measurement of oral malodor as a potential test of the periodontal diseases. Whereas there is a large body of literature (*e.g.* Brening *et al.*, 1939; Fosdick and Piez, 1953; Kleinberg and Westbay, 1990; Yaegaki, this volume) indicating that there may be a positive relationship between oral malodor and periodontal diseases, this has not been quantified in any prospective or case-control studies. In this paper, we address the question: Is there a quantifiable relationship between periodontitis and oral malodor? A detailed account of this work can be found in Bosy *et al.* (1994).

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Diagnostic Tests

It is now beginning to be recognized that early detection of periodontal diseases can lead to improved outcomes. Central to the process of early detection of disease is accurate, differential diagnosis. On the basis of certain clinical features, the American Academy of Periodontology (1989) has advocated the discrimination of at least five different kinds of periodontitis. On the basis of clinical observations there is no difficulty in discriminating some of these diseases. For example, by means of clinical presentation and age, prepubertal periodontitis, juvenile periodontitis and acute necrotizing ulcerative periodontitis are readily diagnosed. However, difficulties arise in resolving rapidly progressive from adult periodontitis. Secondly, within the adult periodontitis group, there are problems in distinguishing what might be the most appropriate forms of initial and maintenance treatments based on clinical course and the natural history of the disease. Therefore, an important rationale for the development of new methods to diagnose and test for the periodontal diseases (and specifically rapidly progressive and recurrent periodontitis), is the reduction of diagnostic uncertainty and the monitoring of these diseases during treatment.

Diseases are initiated at a biological onset, an event that is very difficult, if not impossible, to detect. During the natural history of the disease a point in time is reached when the usual clinical diagnosis is made. Currently, in clinical practice the use of the periodontal probe provides relatively late detection of periodontitis, usually well after periodontal attachment loss has occurred. Based on when this usual clinical diagnosis is made, and in consideration of the availability of appropriate and efficacious treatments, a variety of outcomes are possible: either there can be complete recovery, regeneration and repair of those tissues which are lost or destroyed, or there can be variable levels of permanent disability. Typically, with periodontitis, there may be development of gingival recession, sensitive teeth, loose teeth, or there may be tooth loss. Empirically, it seems wise to promote the development of methods for early diagnosis and thereby shift the time of usual clinical diagnosis to as early a stage as possible in the natural history of the disease. Our specific rationale for using oral malodor as a diagnostic test is based on the metabolism of potentially pathogenic bacteria on teeth, tongue and other sites (Kleinberg and Westbay, 1990). Hopefully, early detection of pathogens will be facilitated by measurement of volatile sulfur compounds (VSC), some of which are known to be components of oral malodor. If a substantive relationship exists between periodontitis and oral malodor, it could be possible to obtain an improved outcome for patients with periodontitis - that is, early diagnosis could lead to recovery or regeneration or repair, as opposed to disability or potential tooth loss.

Currently, there is some legitimate concern among dentists and periodontists about the clinical utility of diagnostic tests and their practical value in patient care. If treatments are available and can be used efficaciously, is there a real need for early diagnosis? However, if periodontitis is of sufficient seriousness to warrant development of methods for early detection, we might also ask: how effective is the ensuing treatment in terms of efficacy, patient compliance and lead time? There is a large data set to indicate that current methods of treating periodontal diseases are reasonably efficacious. But there is also a burden of suffering caused by the treatments themselves, particularly in terms of disability and discomfort after surgical treatment (Matthews and McCulloch, 1993). Currently, we have insufficient evidence to either uphold or negate the need for diagnostic tests in periodontology. Cognizant of the potential pitfalls of periodontal diagnostic tests (McCulloch, 1994) and the need to extend our search for novel approaches, we have examined the use of oral malodor as a diagnostic test.

Volatile Sulfur Compounds as Screening Tests

Can we identify a screening procedure to shift diagnosis to the earliest possible juncture after biological onset? How good is oral malodor as a potential screening test for detection of periodontitis? What are the most appropriate test instruments for examining these questions? Despite the pioneering work of Tonzetich and coworkers in which the gas chromatograph was used to estimate the repertoire of volatile compounds in mouth air (for a review see Tonzetich, 1977), we considered that the gas chromatograph may not be wholly appropriate for examining large numbers of patients. Speed of operation, patient throughput and the need to obtain reproducible data suggested that other methods should be pursued. Instead, we chose a portable, industrial sulfide monitor manufactured by Interscan (Model #1170; Chatsworth, CA). Clinical research experiences obtained at Toronto (Rosenberg *et al.*, 1991a; Bosy *et al.*, 1994) and in Israel (Rosenberg *et al.*, 1991b) indicate that this device is simple, reasonably priced, safe, acceptable to patients and can simultaneously assess the whole mouth rather than measuring multiple separate dental sites as is usually done with periodontal probing. The sampling of whole mouth odor provides an estimate of sulfur-containing volatiles from all teeth and the tongue simultaneously.

In terms of basic measurement theory, we face issues of bias and random errors that strongly affect use of the Interscan monitor and interpretation of the data. For example, the difference between the VSC measurement and the putative gold standard for oral malodor could give an estimate of the bias. Currently, because we lack a gold standard for oral malodor, it has not been possible to estimate bias associated with the Interscan monitor. We have been able to estimate the variability of the monitor due to chance and other random errors by examining replicate measurements made one week apart. The Interscan monitor was used to measure either "steady-state scores" - (the score achieved after the initial peak) or the "peak score" (the highest value found just after the introduction of the probe/drinking straw into the patient's mouth). If the difference between replicate measurements of either the mean steady-state score or the mean peak score made one week apart was plotted against the mean score, ~58% of the steady-state VSC measurements lay within one standard deviation of the mean (Rosenberg *et al.,* 1991a). Thus, the Interscan instrument appears to provide reasonably good replication for repeated, within-patient scores.

There are also significant difficulties that arise in assessing oral malodor with the Interscan monitor. For example, what is the gold standard for assessment of oral malodor? As organoleptic assessment of oral malodor is a subjective process, and is strongly modified by cultural, educational and physiological differences between judges, our initial concern was to relate VSC compounds detected by the Interscan instrument to organoleptic measurements (Rosenberg *et al.,* 1991a,b). As described in detail in the Rosenberg *et al.* (1991a) paper, correlation analysis between logarithmically transformed steady-state VSC scores and organoleptic assessment of mouth air indicated only moderately high associations ($r=0.6$; $p<0.001$).

One of the central difficulties in the application of the Interscan monitor to periodontal diagnosis is to relate VSC measurements to the periodontal disease process. In patients affected by periodontitis in which there is a fairly complex oral microbiota, a large portion of the microbial load is found on the tongue. Conceivably, oral putrefaction at tongue sites may overwhelm that originating from dental sites, thereby reducing the possible efficacy of the oral malodor measurement as a diagnostic tool for periodontal diseases. What is the origin of the oral malodor? Is it simply from the tongue or is it a mixture of compounds from tongue, dental and other oral sites? When we consider the potential contributors to random error and variation that exist in such a complex biological situation, it is remarkable that reproducible measurements can be obtained at all. When VSC scores obtained one week apart were plotted on a continuum, the peak and the steady-state scores exhibited Pearson correlation values of 0.63 and 0.72 respectively (Bosy *et al.*, 1994). What are the origins of these variations? They could be due to the examiner, to the examined, or to the examination procedure itself. Presumably some of this variation is due to biological "noise" in the system being examined. For diagnostic purposes, our specific interest is how disease, or potential disease, may impact on variations that are meaningful and can be interpreted.

VSC and Periodontitis

In the first part of our recent study (Bosy *et al.*, 1994), we examined 127 patients, 107 of whom were recruited from the oral malodor clinic that was started at the University of Toronto. Typically, patients presented to this clinic because of concern about their own malodor. In this study, they represented about 84% of the patient population, while the other 16% of the patient population were drawn from other sources. A variety of measures were made including VSC peak, VSC steady-state, assessments of organoleptically detected oral odor, tongue odor (detected by scraping the tongue), estimates of gingival inflammation (Löe and Silness, 1963) plaque (Silness and Löe, 1964) and organoleptic assessments of floss. Pearson correlation demonstrated that the strongest relationships were between organoleptic assessment of whole mouth odor and the peak and the steady-state VSC scores. Weaker correlations were found between tongue odor and the steady-state scores. Very low, and not statistically significant correlations were found between gingival index and VSC, and between plaque index and VSC. There was no detectable correlation between the presence of pockets >5mm or >7mm and VSC peak or VSC steady-state.

When we examined the tongue for trypsin-like activity using the BANA test (Perioscan; Oral-B), we found that Perioscan scores were related to tongue odor (Chi-Square=16.84; p=0.001) and were also related more weakly to floss odor (Chi-Square=6.52; p=0.01). Thus, positive Perioscan scores were related to floss odors. We also performed some assessments of oral wetness using sialopaper strips and a modified Periotron (Interstate Drug, Amityville, NY) but were unable to find a statistically detectable association between oral wetness and the presence of organoleptic malodor.

Pearson correlation assumes a linear relationship between the two parameters of interest and is not always the most appropriate analysis for assessing associations, particularly with ordinal data sets. Whereas there was an apparent relationship between VSC and organoleptic assessments, there was no detectable relationship between VSC and commonly used measures of periodontal health (*i.e.*, Gingival Index, Plaque Index or the presence of pockets >5mm or >7mm). As dichotomous analyses may be more appropriate for assessing associations involving measures of periodontal health, we performed a dichotomous analysis of periodontitis/no periodontitis as defined by the presence or absence of pockets <5mm as well as attachment loss below the cemento-enamel junction. Halitosis/no halitosis was evaluated on the basis of an organoleptic score above or below a value of 3 on an ordinal scale. This threshold level was computed from a previous study (Rosenberg *et al.*, 1991a). Within each cell there were 23 patients in the periodontitis/halitosis group, 52 patients in the no periodontitis/halitosis group, 14 patients in the periodontitis/no halitosis group, and 38 patients in the no periodontitis/no halitosis group. Chi-square analysis showed no significant association (Chi-Square=0.2; p=0.65) and the odds ratio was 1.2, indicating no potentially useful diagnostic relationship between measurement of VSC and detection of periodontitis. For the four groups, periodontitis/halitosis, no periodontitis/ halitosis, periodontitis/no halitosis, and no periodontitis/no halitosis, the age ranges, VSC scores, whole mouth organoleptic scores and tongue pHs were similar between groups (except of course for the periodontal measurements, which was how the periodontitis/no periodontitis groups were established). Inspection of these individual values indicated that our separation into the four groups was consistent with reality and was a reasonable division of patients into the groups.

As the inclusion of oral malodor clinical patients may have been a major confounder in this analysis, a separate analysis was undertaken in which these patients were deleted. Application of Fisher's exact test to the same dichotomous analysis demonstrated no statistically significant association (p=0.29). We conclude therefore that this result was not simply a spurious lack of relationship that arises from an abnormal group of patients but was real.

We performed multiple stepwise regression on log-transformed VSC scores to obtain a distribution of VSC measurements that more closely approximated a Gaussian distribution and to asssess further the relationship of VSC and organoleptic measurements. There was a linear relationship between oral odor or tongue odor and steady-state VSC (p=0.001) but not with floss odor.

Chlorhexidine Effect on VSC

In the final part of the study, patients were provided with 0.2% chlorhexidine as a rinse that was suggested to be used three times a day. We were not able to directly check compliance; it was assumed that patients were actually using the rinse as instructed. At appointment 1 and appointment 2, we obtained replicate scores. Between appointments 2 and 3, patients used the chlorhexidine rinse for one week. Chlorhexidine rinsing for one week reduced tongue pH by 0.7 pH unit, a 400% increase in the concentration of hydrogen ions. Associated with the reduction of tongue pH was a sharp reduction of oral odor (~65%). There was also a ~57% reduction of nasal odor (detected organoleptically) and a ~40% reduction of tongue odor. Gingival index was reduced only 17%. As expected, there were no changes in the number of pockets >7mm. Thus, in this experimental section of the investigation in which chlorhexidine was used to pharmacologically reduce oral malodor, the Interscan monitor detected the expected change in VSC production but this reduction was not strongly linked to short-term improvements in periodontal health.

In contrast to the study of Bosy *et al.* (1994), both Kozlovsky *et al.* (1994) and Yaegaki (this volume), have provided data showing a quantitative relationship between oral malodor and periodontal diseases. Further, Yaegaki reviews a large number of papers which describe a relationship between periodontal diseases and either VSC or oral malodor. However, we cannot identify in these references any specific attempt to differentiate between the various types of periodontal diseases and in particular to quantitatively establish a relationship between the levels of individual VSC components and the severity of *periodontitis*. Instead, we note that Yaegaki has identified a very compelling relationship between tongue coating and VSC. He also reports that tongue coating is related to periodontal disease status and that the *ratio* of methyl mercaptan to hydrogen sulfide increases with maximum probing depth. Evidently, there is no strong positive relationship between single measures of VSC (*e.g.* Interscan measurements) and the severity of periodontitis.

Conclusions

Collectively, these data support a number of points. First it is possible to make reproducible measurements of oral VSC with the Interscan monitor. Currently, we do not know what the relationship of the monitor is to any kind of a gold standard, and establishing a relationship between known standards and oral VSC needs to be done in the future if the Interscan monitor is to be used more widely. Volatile sulfur measurements in general made with the Interscan monitor correlate reasonably well with organoleptic assessments. The Interscan monitor is sensitive enough to detect an expected reduction of oral malodor that is produced by chlorhexidine. However, the major finding in this study is that we were unable to use the VSC monitor or organoleptic assessments of oral malodor as a screening test for the presence of *periodontitis*. Indeed, sharp reductions in VSC and oral malodor can be obtained independent of improvements in periodontal health.

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8

Oral Malodor in the General Population of Japan

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The aims of this study were to obtain basic data on oral malodor in the general population and to examine the relationship between oral malodor and oral health status. People (N=2,601) aged 18 to 64 years who live in Fukuoka Prefecture, Japan, were measured for their oral malodor using a portable sulfide monitor (Interscan Corp., Model RH17A, Chatsworth CA) by two dentists. In addition, dental (DMF) and periodontal measurements (PMA, CPITN and attachment loss), dental plaque and tongue coating status were assessed by four dentists. Interviews for subjects concerning oral health behavior, smoking habits and medical history were conducted prior to clinical examinations. Subjects measured in the morning were divided into those checked within 2.5 hours following oral activities, or following more than 2.5 hours (groups M2.5h> and M2.5h<, respectively); subjects measured in the afternoon were divided into those checked within 2.5 hours following oral activities, or following more than 2.5 hours (groups A2.5h> and A2.5h<, respectively). Data were also analyzed according to gender and age group. In each gender and age group, the measurement values of oral malodor were

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highest in the M2.5h< group (61.0 ppb in average), followed by A2.5h< (54.8 ppb) and A>2.5 (41.6 ppb). Values tended to increase with increasing age in both males and females in each measurement period, especially within 2.5 hours after lunch (A2.5h>). Statistical significant correlations were observed between oral malodor and periodontal conditions as well as tongue coating status. However, there were no significant correlations between oral malodor and DMF, plaque, smoking habits, or self-evaluation. In conclusion, these results suggest that oral malodor might be caused mainly by tongue coating in the younger generation and by periodontal diseases together with tongue coating in older cohorts in the general population.

Introduction

Oral malodor has been a subject of considerable public interest in Japan as well as other industrialized countries for the last few decades. However, assessment of oral malodor has been excluded from oral examination procedures because of the lack of objective measuring methods for field surveys. Since Rosenberg *et al*. (1991a,b) reported a portable sulfide monitor with reproducibility and sensitivity for measuring volatile sulfide compounds (VSC) in the oral cavity which are associated with oral malodor, it has become possible to assess oral malodor of people at the community level.

The purpose of the present survey was to obtain basic data on oral malodor at the community level in order to introduce an oral malodor screening system for oral health surveys and regular dental check-ups at schools, work places and health centers, as well as for planning and implementation of an oral health program for the general population.

Materials and Methods

In the present survey, VSC of each subject were measured with a portable sulfide monitor, Model RH17A (Interscan Corporation, Chatsworth, CA). A disposable 60 mm glass tube connected with a plastic tube was attached to the air inlet of the monitor. Each subject was instructed to bring his slightly-opened mouth over the tube so that it extended about 4 cm into the oral cavity and to breathe through his nose during the measurement. The sample of mouth air passed through a sensor, which registered the level of sulfides. Maximum level attained was determined in parts per billion (ppb) sulfide equivalents by direct readings from the digital display of the monitor.

Four examiners assessed subjects' oral health conditions, including DMFT, periodontal conditions by PMA index (Massler and Schour, 1949), CPITN (Community Periodontal Index of Treatment Needs) and attachment level, basically according to the WHO guidelines (Ainamo *et al.*, 1982; WHO, in press). They also assessed oral hygiene status by plaque index (Silness and Löe, 1964) and tongue coating status according to distribution area of the tongue. Interviews for subjects were carried out concerning their oral health behavior, including frequency of toothbrushing per day, smoking habits (daily smoking or not) and medical history, and the time when subject had final oral activity before clinical examinations. Subjects were also asked whether or not, and how often, they noticed their own oral malodor. Their answers were subsequently recorded as self-estimates according to the following index: 0 - having no malodor, or seldomly having malodor; 1 - sometimes having malodor; and 2 - often having malodor. Before and during the surveys, the examiners were calibrated using volunteer dental students and dental patients. Intra- and inter-examiner reliability was generally in the range considered to be good as assessed by Kappa statistic (Landis and Koch, 1977).

In 1992, an epidemiological survey on oral malodor was carried out in Fukuoka Prefecture, situated about 1,000 km from Tokyo to the south-west. All subjects were local government employees engaged in various kinds of work: office workers, teachers, medical doctors, engineers, garbage collectors, etc. About half of them live in urban areas and the other half in rural areas. So far, data from 2,601 people, aged 18 to 64 years, have been collected and stored in our department.

Table 1 shows the distribution of subjects by age group, gender and time measured. The subjects were divided according to four time categories. Members of the first group were examined within 2.5 hours of their final oral activity of the morning, such as breakfast, drinking tea, toothbrushing, etc. (earlier morning group; M2.5h>). The second group was examined following more than 2.5 hours since previous oral activities in the morning (later morning group; M2.5h<). Similarly, the third and fourth groups represent examinations conducted within 2.5 hours following oral activities in the afternoon (A2.5h>) and following more than 2.5 hours since previous oral activities in the late afternoon (A2.5h<).

Table 1
Number of subjects by age group, gender, and time of measurement

Age (yrs)	Gender	M2.5h>	M2.5h<	A2.5h>	A2.5h<	Total
15-24	Males	21	28	94	15	158
	Females	12	12	93	11	128
25-34	Males	67	85	183	35	370
	Females	26	27	114	20	187
35-44	Males	147	188	200	51	586
	Females	67	60	183	18	328
45-54	Males	73	118	144	52	387
	Females	42	61	110	15	228
55-64	Males	44	50	49	7	150
	Females	17	14	39	9	79

Results

Figure 1 shows the mean values of VSC in the 15-24 year-old group according to gender and time of measurement. In this age group, the highest values were observed for both males and females in the later morning. For males, the average was 70 ppb, and 58 ppb for females. The lowest values were observed in the earlier afternoon: 33 ppb for males and 36 ppb for females. Significant differences were found between the later morning group and the other three groups for males, and between the earlier afternoon group and the later groups in both the morning and afternoon, for females.

Figure 2 shows the mean values of VSC in the 25-34 year-old group according to gender and time of measurement. A similar pattern was observed for the 15-24 year-old group. The VSC value of males measured in the later morning group was significantly higher than that of males measured in the earlier groups of both the morning and afternoon. Values in males measured in the earlier morning group were significantly higher than those measured in the earlier afternoon group. For female subjects, there were no significant differences observed between groups.

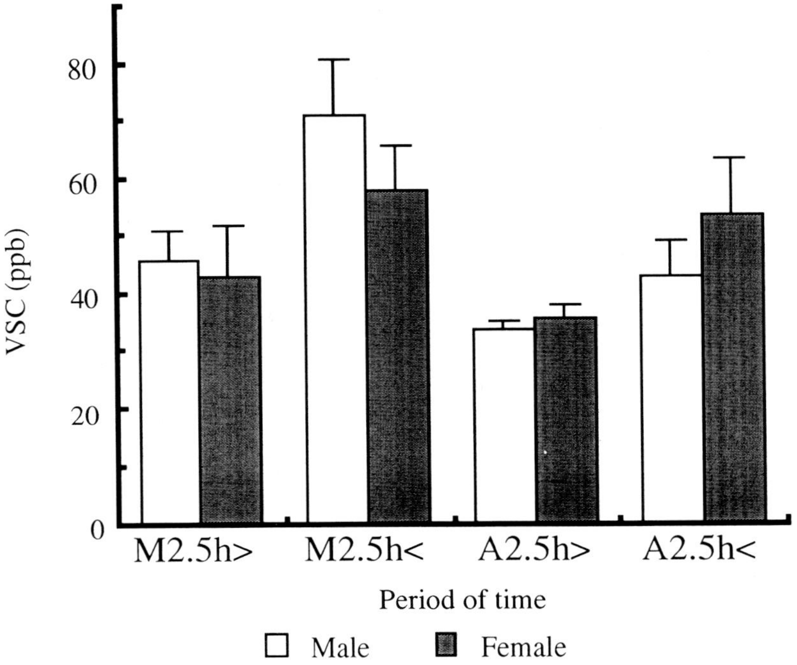

Figure 1. Mean values of volatile sulfide compounds (VSC) in 15-24-yr-old grouped by gender and time of measurement.

Figure 3 shows the mean values of VSC in the 35-44 year-old group according to gender and time of measurement. The VSC value of males in the earlier afternoon group was significantly lower than that in the other 3 groups. For females, the VSC value measured in the later morning group was significantly higher than those in the other three time groups.

Figure 4 shows the mean values of VSC in the 45-54 year-old group according to gender and time of measurement. In the male subjects, VSC values in time groups measured beyond 2.5 hours in the morning and afternoon were significantly higher than in the earlier groups both in the morning and afternoon. In the female subjects, the VSC value in the earlier afternoon group was significantly lower than that in the earlier morning group.

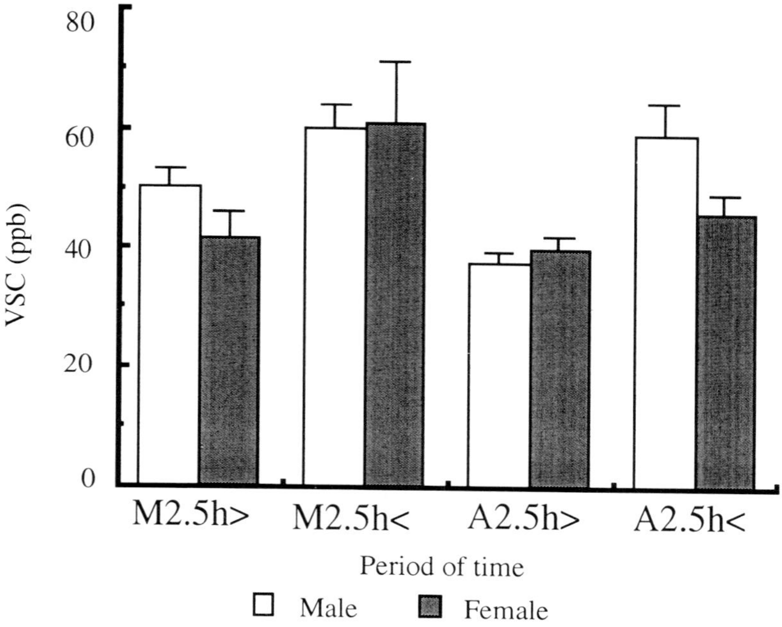

Figure 2. Mean values of VSC in 25-34-yr-old grouped by gender and time of measurement.

Figure 5 shows the mean values of VSC in the 55-64 year-old group according to gender and time of measurement. For males, the VSC value in the later morning group was significantly higher than that in the earlier groups both in the morning and afternoon. For female subjects, the sample size in all time groups was too small to interpret the data.

Figure 6 shows the mean values of VSC measured within 2.5 hours after final oral activity in the morning according to gender and age group. Mean values of VSC in males tended to be higher than those in females in each age group except for the 45-54 year-old group. A significant difference was observed between males and females only in the 55-64 year-old group. No significant differences of VSC values were shown between age groups both in males and females, except for that in the 55-64 year-old females.

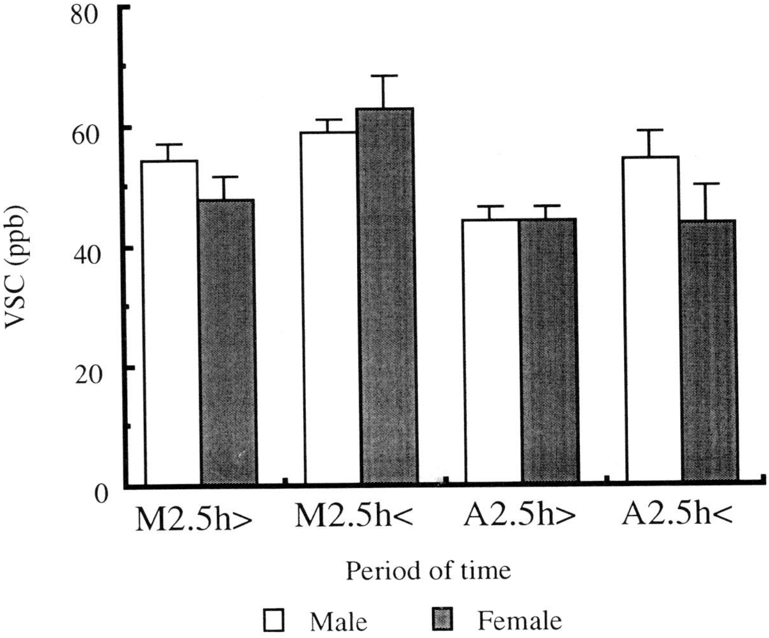

Figure 3. Mean values of VSC in 35-44-yr-old grouped by gender and time of measurement.

Figure 7 shows the mean values of VSC measured beyond 2.5 hours after final oral activity in the morning divided according to gender and age group. There were no significant differences between gender in all groups and also among age groups in both males and females.

Figure 8 shows the mean values of VSC measured within 2.5 hours after final oral activity in the afternoon according to gender and age group. Mean values of VSC showed higher levels with increasing age. Significant differences were observed between the 35-44, 45-54, 55-64 year-old groups and the 15-24, 25-34 year-old groups in males, and 35-44, 45-54, 55-64 year-olds and 15-24 year-olds in females. Mean values of males were very similar to those of females in each age group.

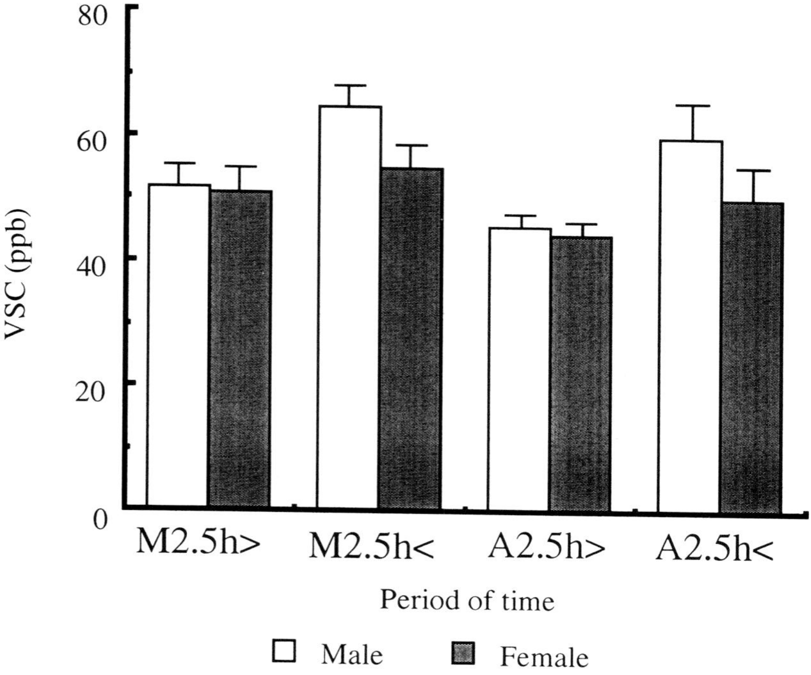

Figure 4. Mean values of VSC in 45-54-yr-old grouped by gender and time of measurement.

Figure 9 shows the mean values of VSC measured beyond 2.5 hours after final oral activity in the afternoon according to gender and age group. There were no significant differences between age groups in either males or females. The mean VSC value of males was significantly higher ($p<0.05$) than that of females only in the 25-34 year-old group.

Table 2 shows the Pearson correlations between VSC values and parameters of oral malodor in the later morning group, when the highest mean VSC values were observed. Very few people had a loss of attachment of 4 mm or over, and also very few were using medication, so that both these parameters were excluded from this analysis. Pearson correlation analysis consistently

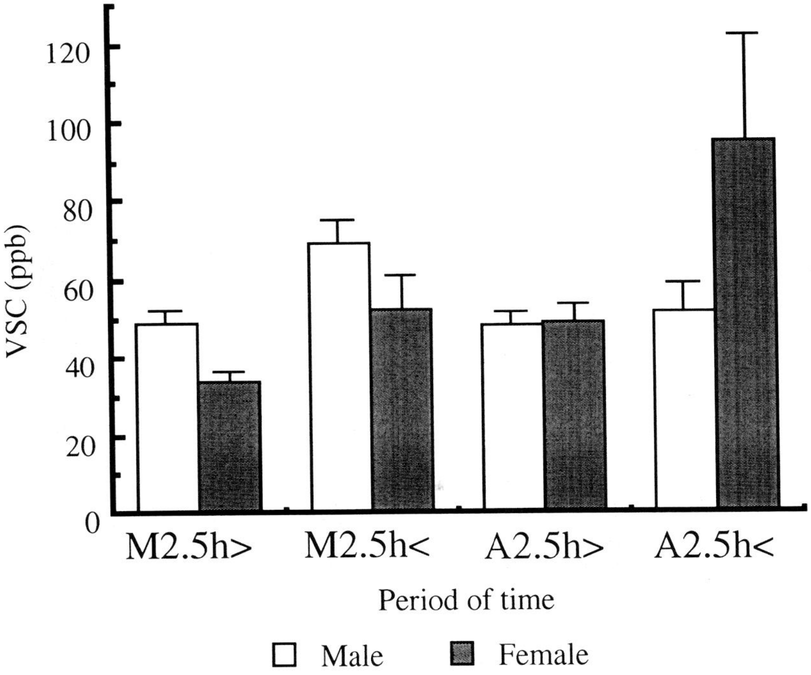

Figure 5. Mean values of VSC in 55-64-yr-old grouped by gender and time of measurement.

demonstrated an association between VSC values and tongue coating in every age group. In addition, an association between VSC and periodontal conditions was observed. For the 55-64 year-old group, higher correlation coefficients were found. However, we could find no correlation between VSC and the number of decayed teeth, or frequency of toothbrushing in any age group.

Correlations between VSC values and dental parameters in the earlier afternoon group, when the lowest mean VSC values were obtained, are presented in Table 3. Similar results were shown but lower correlation coefficients were generally found as compared with those in the former group (Table 2). We could see a weak association between VSC and tongue coating, and between VSC and periodontal conditions (Table 3).

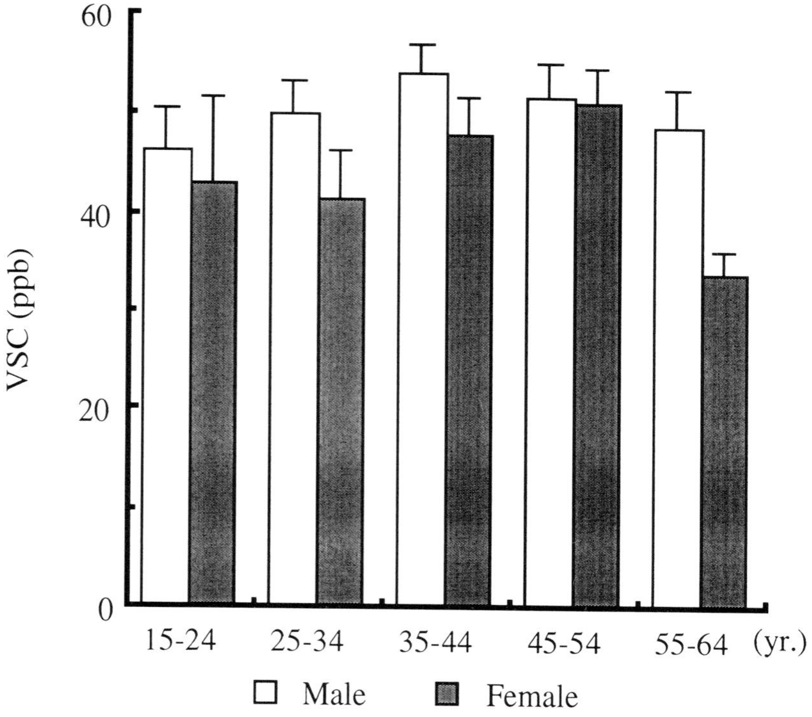

Figure 6. Mean values of VSC by age group within 2.5 hours after oral activities in the morning.

Table 4 shows the mean values of VSC in the groups, divided according to smoking habit or self-estimation by the time period of measurement. The mean values of VSC tended to be higher in the non-smoking group than in the smoking group, with significant differences ($p<0.05$) in the later morning and earlier afternoon. In both earlier and later morning measurement groups, the VSC mean values of persons who sometimes felt their own oral malodor were significantly higher ($p<0.05$) than those of persons who never or seldom felt it.

Table 2

Pearson correlations between VSC and dental parameters in subjects checked in the morning, measured following at least 2.5 hours since prior oral activity, according to age group

VSC	15-24 N=40	25-34 N=112	35-44 N=248	45-54 N=179	55-64 N=64
Tongue coating	0.559***	0.462***	0.411***	0.367***	0.602***
CPITN	0.281	0.323***	0.246***	0.319***	0.699***
PMA Index	0.337*	0.256**	0.227***	0.226**	0.590***
Plaque Index	0.347	0.085	0.003	0.144	0.314*
Frequency of brushing	0.081	-0.147	0.104	0.055	-0.129
Decayed teeth	0.038	-0.091	-0.143*	-0.043	-0.089

*Significant at $p<0.05$
**Significant at $p<0.01$
***Significant at $p<0.001$

Discussion

The purpose of this survey was to ascertain basic data from the general population during their daily life, rather than a given patient population. Thus, measurements of volatile sulfide compounds (VSC) in each subject were carried out at any time between 9:00 am and 5:00 pm. Therefore, interpretation of the data calls for caution because there is a large variation in the number of subjects in groups which are divided according to age and measuring time period. Subject groups were classified by 2.5 hours after final oral activity in both morning and afternoon based on previous reports (Tonzetich and Ng,1976; Tonzetich, 1978). In those studies, hydrogen sulfide and methyl mercaptan levels were depressed for at least 2 hours after meals, and tongue and toothbrushing resulted in reduction of VSC for a period of 2 to 3 hours. In our cross-sectional survey, indeed, the mean values of VSC before lunch were higher by 30% than after lunch and were higher in groups for which a longer time had passed since oral activities in both morning and afternoon.

Table 3
Pearson correlations between VSC and dental parameters in subjects checked in the morning, measured following less than 2.5 hours since prior oral activity, according to age group

VSC	15-24 N=187	25-34 N=297	35-44 N=383	45-54 N=254	55-64 N=88
Tongue coating	0.499***	0.317***	0.270***	0.451***	0.293***
CPITN	0.282***	0.287***	0.294***	0.429***	0.261***
PMA Index	0.138	0.159**	0.140**	0.262***	0.400***
Plaque Index	0.177*	0.147*	0.101*	0.148*	0.149
Frequency of Brushing	-0.143	-0.016	-0.1039	0.018	-0.052
Decayed teeth	0.150*	-0.003	-0.023	0.018	0.042

*Significant at p<0.05
**Significant at p<0.01
***Significant at p<0.001

Rosenberg *et al.* (1991a) suggested that sulfide measurements with the monitor as well as organoleptic scores of malodor were positively related to the age of the subject. This age-related increase was similar in both genders. The present survey data also showed that the mean values of VSC by age group tended to increase with age. However, a significant difference was observed only in the period of time within 2.5 hours after oral activities in the afternoon.

Some scientific studies have shown that periodontal disease and tongue coating constitute the major source of VSC and are responsible for the offensive odor (Tonzetich and Ng, 1976; Tonzetich, 1978; Kostelc *et al.*, 1984; Yaegaki and Sanada, 1992a,b). The tongue coating is comprised of epithelial cells derived from the oral mucosa, microorganisms and leukocytes from periodontal pockets. Malodorous sulfur compounds in mouth air were

Table 4
Mean values of VSC in subject groups according to smoking habit or self-estimation, and as a function of time of measurement

	M2.5h>		M2.5h<		A2.5h>		A2.5h<	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Smoking habits								
Non-smoking	308	40.3 (26.7)	397	51.1 (31.6)	856	34.1 (14.7)	150	45.7 (31.4)
				*		*		
Smoking	208	38.9 (18.9)	246	45.1 (26.7)	353	31.5 (21.4)	83	40.7 (19.8)
Self Estimation								
None/seldom	305	37.4 (22.4)	397	46.3 (28.0)	754	32.7 (20.1)	154	45.9 (30.3)
		*		*				
Sometimes	196	43.2 (26.1)	236	52.3 (31.2)	420	34.1 (19.1)	71	39.0 (22.0)
Often	15	42.7 (15.6)	10	67.4 (54.7)	35	37.3 (20.0)	8	51.0 (20.4)

*Significantly different ($p<0.05$)

found to increase quantitatively in subjects with gingivitis as compared with control subjects (Kostelc *et al.*, 1984). Salivary volatile production increased with deterioration in gingival health and, conversely, decreased with maintenance of optimal periodontal conditions (Kostelc *et al.*, 1984). Tonzetich (1978) observed a positive correlation between the severity of periodontitis (periodontal pocket depths) and VSC content in mouth air. Yaegaki and Sanada (1992a) also confirmed that not only microorganisms, but also the tongue coating and gingival fluid were factors which enhanced VSC production in patients with periodontal disease. In this survey, Pearson correlation analysis demonstrated an association between VSC and tongue coating in every age group. Similarly, an association between VSC and periodontal conditions was observed. For the older age groups, higher correlation coefficients were found. These findings are in agreement with the results reported by Rosenberg *et al.* (1991a).

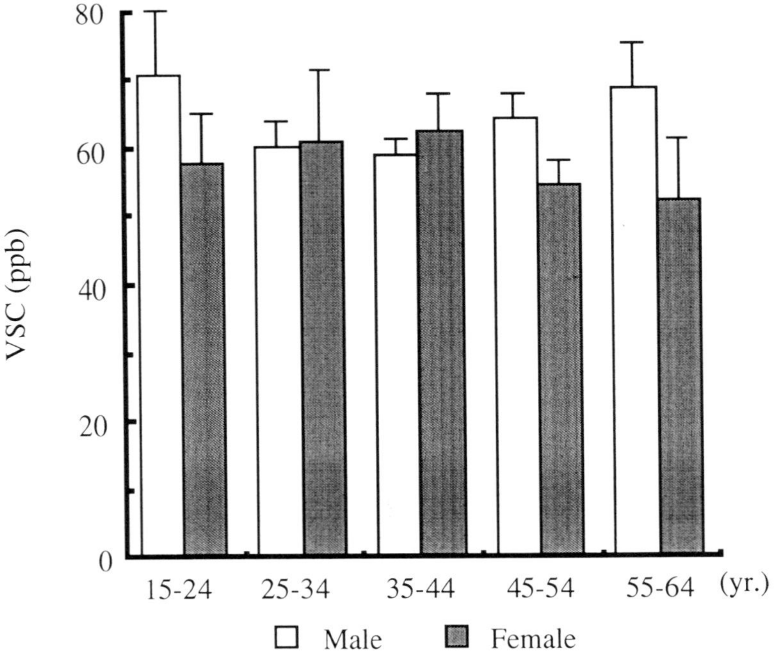

Figure 7. Mean values of VSC by age group beyond 2.5 hours after oral activities in the morning.

In our previous survey on periodontal conditions in the Japanese population measured by CPITN, there were significant differences between males and females with respect to pocket depths of 4 mm or more and 6 mm or more (Miyazaki *et al.*, 1989). However, no significant difference was shown in VSC between genders as found in an early study (Sulser *et al.*, 1939). This might suggest that for people in the general population with relatively low levels of VSC, oral malodor is not caused by periodontal diseases alone but by a combination of several factors, including periodontal conditions, tongue coating, salivary characteristics and salivary flow rate. Furthermore, the relative disease activity of the periodontal sites themselves may be more important than the mere presence of deeper periodontal pockets.

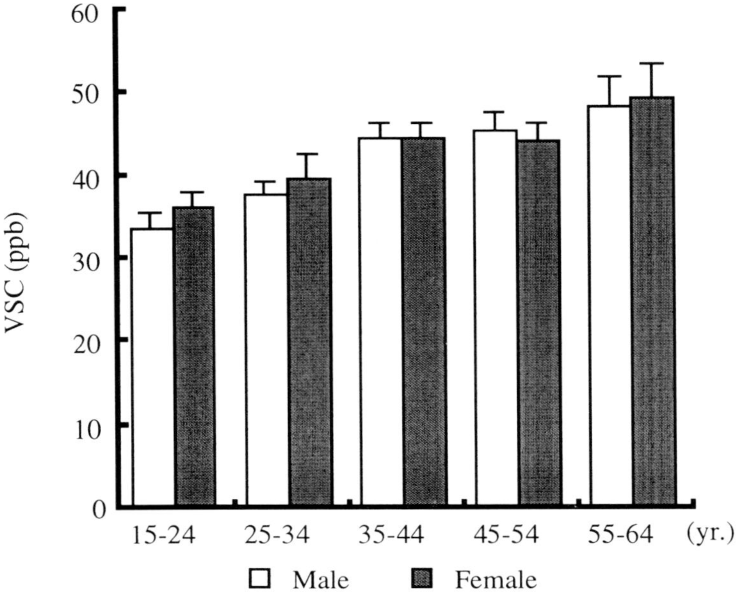

Figure 8. Mean values of VSC by age group within 2.5 hours after oral activities in the afternoon.

On the other hand, the finding that non-smokers may have higher levels of VSC than smokers, might suggest that smoking reduces bad breath, perhaps by antibacterial activity. Whereas tobacco smoke itself contains VSC (Stedman, 1968), its contribution to overall VSC in persons who did not smoke just before examination is probably very low.

Figure 10 illustrates the distribution of the combined VSC values of subjects between 18 and 64 years of age as measured at different times. The consistently higher VSC values are distributed in subjects measured in the later morning group. From the left curve to the right, respectively, mean VSC values are 42 ppb, 50 ppb, 55 ppb, and 61 ppb. In each instance, mean VSC values are situated at around the 65 percentile. If we take 75 ppb as a socially acceptable level suggested by Yaegaki and Sanada (1992c), 8%, 15%, 18%, and 25% of subjects measured in the earlier afternoon, earlier morning,

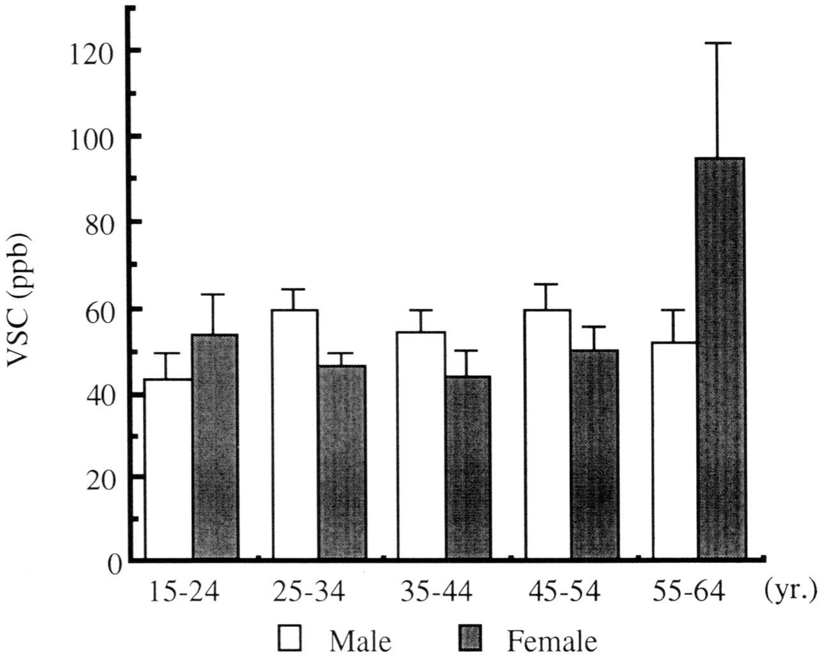

Figure 9. Mean values of VSC by age group beyond 2.5 hours after oral activities in the afternoon.

later afternoon, and later morning group, respectively, might have oral malodor. Moreover, these results might suggest that approximately 10% of the population does not have oral malodor in the afternoon but has malodor in the late morning.

In conclusion, different VSC values were observed as a function of the time passed since oral activity for each age group. The VSC values of males tended to be higher than those of females. However, no significant differences were shown between males and females in most of the measurement groups. VSC values obtained tended to be higher with increasing age. Correlation analysis demonstrated an association between VSC and tongue coating, as well as between VSC and periodontal conditions. However, no association

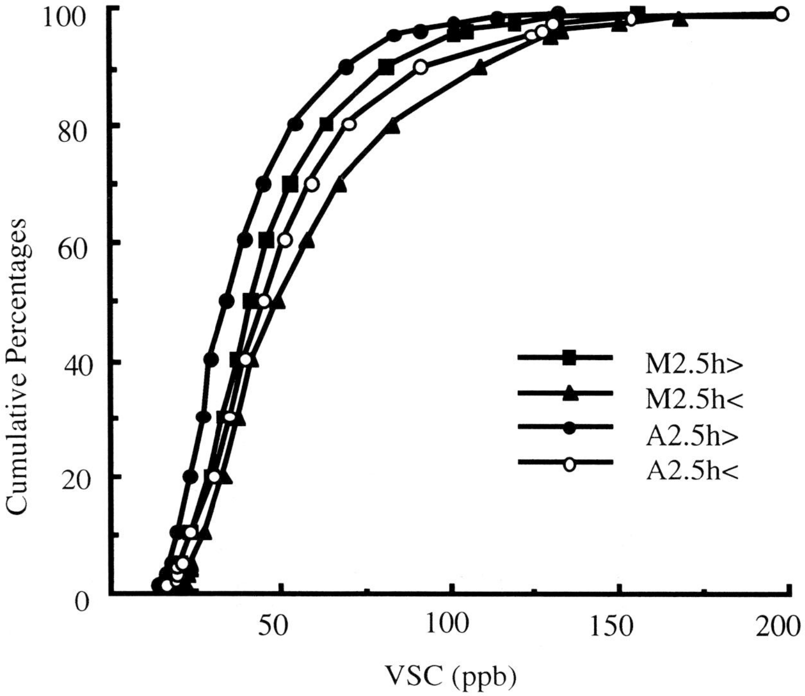

Figure 10. Distribution of VSC and cumulative percentages by different time measured in 18-64-yr-old subjects.

was found between VSC values and the number of decayed teeth, toothbrushing or smoking habits. Up to 25% of subjects exhibited VSC values higher than the suggested socially acceptable limit of 75 ppb.

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9

Experiences of an Israeli Malodor Clinic

Mel Rosenberg[1,2*] and Efrat Leib[2]

Oral malodor is an intimate human problem which crosses over many scientific disciplines and has many nuances. Whereas laboratory research is of great importance in understanding various aspects of oral malodor, clinical experience is also a prime factor. For the past three years, we have provided an oral malodor consultation service in a medical center in Tel Aviv. The present report summarizes some of our findings, with particular reference to 308 individuals who sought our advice between February and December, 1992.

Individuals who presented at the clinic between February and December, 1992 (N=308) became aware of the clinic primarily through (i) articles in the local press and in radio interviews; (ii) referrals from dentists and physicians; and (iii) individuals who alerted spouses and other family members. Sixty percent (N=185) were women.

Consultations were performed in the following manner. First, an interview regarding general health and medical history was conducted, with emphasis on oral hygiene practices, and any other conditions which might conceivably

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Table 1
Mean Odor Scores (± Standard Deviation) of Self-Estimation and Odor Judge Scores of Whole Mouth, Tongue Dorsum Anterior (Wrist-licking Test), and Tongue Dorsum Posterior (Plastic Spoon Test) in an Israeli Population

Category	Men	Women
Self-estimation	3.40 ± 0.92[a]	3.69 ± 1.04[b]
Odor judge, whole mouth	2.62 ± 0.97[c]	2.14 ± 0.95[d]
Odor judge, tongue dorsum anterior	2.70 ± 1.29[e]	1.93 ± 1.32[f]
Odor judge, tongue dorsum posterior	2.94 ± 1.37[g]	2.70 ± 1.29[h]

[a] Significantly different from self-estimation by women ($p<0.001$) and odor judge mouth estimation of men ($p<0.001$), N=49.

[b] Significantly different from odor judge mouth estimation of women ($p<0.001$), N=89.

[c] Significantly different from odor judge mouth estimation of women ($p<0.001$), N=114.

[d] N=181

[e] Significantly different from odor judge tongue dorsum anterior estimation of women ($p<0.001$), and odor judge tongue dorsum posterior estimation of men ($p<0.001$), N=112.

[f] Significantly different from odor judge tongue dorsum posterior estimation of women ($p<0.001$), N=168.

[g] Significantly different from odor judge tongue dorsum posterior estimation of women ($p<0.001$), N=137.

[h] N=154.

lead to malodor (primarily involving nose, throat, and allergies). The subjects were then asked to present a history of the complaint itself, including a brief description of the perceived effect on their lives. Subjects were asked to estimate their own malodor level, prior to measurement, on a 0-5 intensity scale (preconception) in half-integer increments. Subjects were then judged organoleptically on the same 0-5 intensity scale (Rosenberg *et al.,* 1991a), in several manners. First, whole mouth malodor was assessed, as described in previous studies (Rosenberg *et al.,* 1991a,b). Tongue dorsum (anterior) odor was assessed by asking the subject to extend his/her tongue and lick his/her wrist in a perpendicular fashion (Kozlovsky *et al.,* 1994). The odor level was assessed after five seconds. Tongue dorsum (posterior) odor was assessed by scraping this area with a small, disposable, plastic spoon, and assessing the odor on the spoon after five seconds. Nasal odor was distinguished from oral odor (Frankel, as cited by Prinz, 1930) by asking the subject to close his/her mouth and to exhale through the nose.

Mean values for self- and odor judge assessments are summarized in Table 1. Whereas women scored significantly higher than men in rating their own malodor ($p<0.001$), men were assigned higher mean scores in all odor judge tests ($p<0.001$). Self-estimates were significantly higher than odor judge scores for both men and women ($p<0.001$). Posterior tongue dorsum odor judge scores were consistently higher than anterior tongue dorsum levels ($p<0.001$).

As shown in Figure 1, whole mouth odor judge scores were distributed normally. Distributions of tongue dorsum scores (anterior and posterior) are compared in Figure 2. The frequency of very high scores was greater in the case of the tongue dorsum posterior as compared with anterior levels. Furthermore, low anterior scores were more frequent than low posterior scores.

Distributions of self-estimates for men and women are shown in Figures 3 and 4, respectively. In the case of women, 25 out of 88 scored themselves as a "5" (worst conceivable odor), whereas the odor judge assessments of these 25 individuals did not exceed 3.5 (Figure 5).

Among the many types of odors which may present from the mouth and nose, several kinds of odors were found to predominate. These included (i) odor of subgingival putrefaction, often encountered during dental flossing or scaling; (ii) odor of the tongue dorsum anterior, as sampled by licking

Table 2
Typical vs. Non-typical Odors from Four Loci

Smell emanating from	No. of typical odors	No. of atypical odors	Percent typical odors
Whole mouth	252[a]	43	85
Nose	105	29	78
Tongue dorsum anterior	266	3	99
Tongue dorsum posterior	243	3	99

[a] Characteristic of odors indicating oral etiology.

the wrist;(iii) odor of the tongue posterior, as sampled by the spoon method; (iv) odor of nasal origin (although this odor has been termed "ozena" in previous reports [*e.g.* Spouge, 1964], this term should be reserved for description of frank nasal infections [see also Finkelstein, chapter 11, this volume]); (v) smokers' breath; and (vi) denture odor.

Table 2 describes the frequency of finding four such "typical" odors, as compared with atypical odors deriving from the respective sites. Tongue odors appeared to be typical in practically all instances. In the case of whole mouth and nasal malodors, in 85% and 78% of the cases, respectively, the odors seemed typical in nature.

Although the large majority of cases summarized here were deemed to involve malodors of oral origin, nasal odors were not uncommon (Table 2). Two cases are worthy of mention. In the first, a 28-year-old woman from abroad was referred by a dentist, and claimed to have bad breath, despite an excellent dentition. Three types of odors were concomitantly detected: (i) oral odor; (ii) cigarette odor, and (iii) atypical nasal odor. The woman was subsequently referred to an otolaryngologist who found a calicified object in the nasal passage which had been previously overlooked. The object was removed by surgery and proved to be a children's bead, presumably left up her nose for some 25 years.

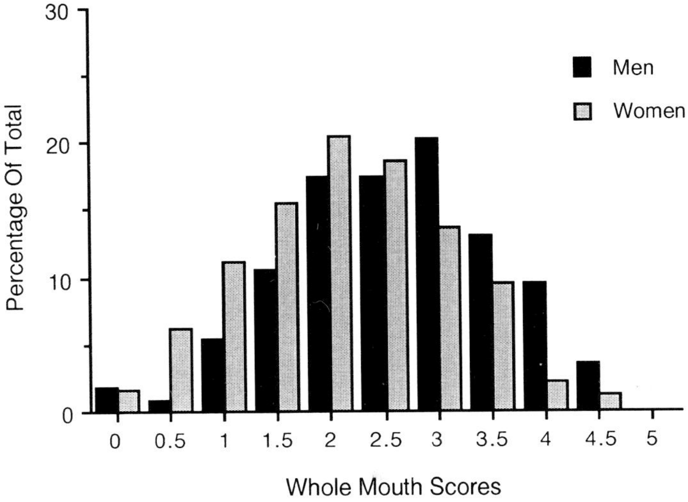

Figure 1. Distribution of odor judge scores.

In the second case, a 19-year-old male, with Kleinfelter Syndrome (XXY), microcephalus and severe mental retardation, was brought to the malodor clinic. The patient was referred to us by a hospital dental clinic, where he had been treated for the past 11 years, and to which his parents had initially brought him two months earlier, with the complaint of oral malodor. During this period, he had been to see various specialists with no improvement in the presenting odor. The odor was typically nasal in its character, yet appeared to emanate from his entire body. However, closer examination revealed that the odor was strongest from the nasal region, but also appeared on the hair, hands and clothes. Regions of the body with which the patient's hands did not come into contact (*e.g.*, the back, the ankles) were relatively free of odor. It was thus apparent that the odorous, purulent discharge had been spread from the nose by hand to other parts of the body, simulating a generalized body odor (Syfert, 1979). A subsequent otolaryngologic examination revealed putrefied paper tissue which was removed, with concomitant disappearance of the presenting malodor.

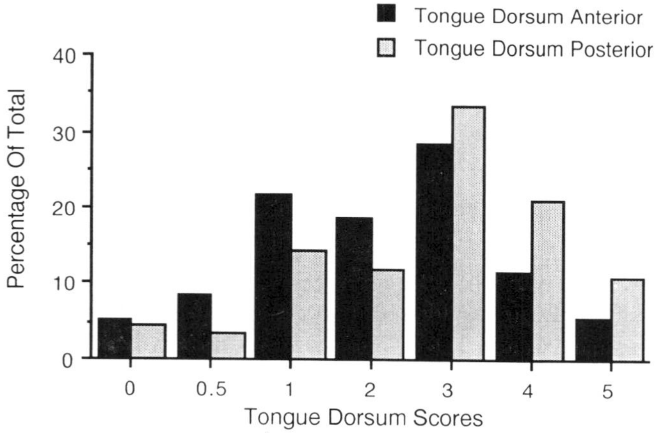

Figure 2. Distribution of odor judge scores of tongue dorsum anterior and tongue dorsum posterior samples.

Discussion

Bad breath is a condition which affects a large proportion of people around the world. In the clinic, we have encountered men and women from practically all walks of life, including teachers, bankers, lawyers, judges, dentists, politicians and airline pilots. Because of the importance of the mouth in self-esteem and self-image (Fisher, 1986), one's perception of suffering from oral malodor may have serious repercussions. Some of the statements made by the subjects reflect the severity of the perceived problem: "It ruins my life"; "I feel defiled"; "I sleep separately from my husband"; "It's like having cancer". Indeed, suicide cases linked to perceived bad breath have been reported (Crohn and Drosd, 1942; Yaegaki, this volume).

The data presented here provide further support for the premise that a significant number of individuals tend to grossly overestimate their own oral malodor levels (Spouge, 1964; Rosenberg *et al.*, 1995; Iwakura *et al.*, 1994). Subjects often remark of being able to measure their own bad breath by various techniques, including cupping hands over mouth and nose, licking

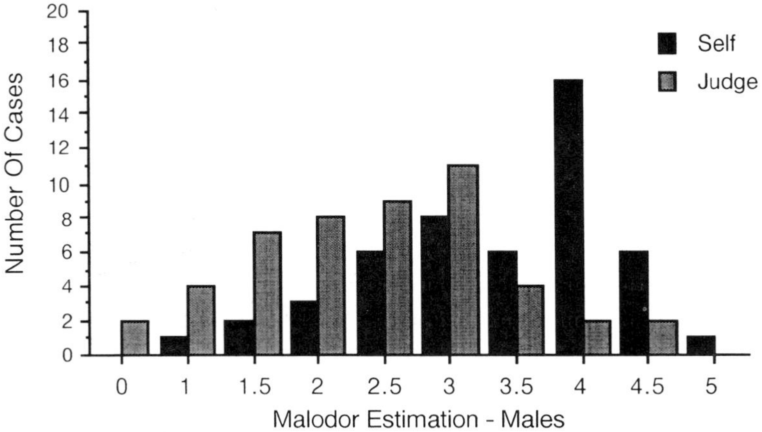

Figure 3. Odor judge whole mouth scores vs. self-estimation in males.

hands, smelling saliva, smelling the telephone mouthpiece, rubbing fingers across the gums, and breathing beneath a blanket. These accounts formed the basis for a recent study on self-estimation of oral malodor levels (Rosenberg *et al.*, 1995). Subjects (N=52) were asked to score their own malodor by cupping hands over mouth ("whole mouth malodor"), licking their wrist ("tongue malodor") and expectorating into a petri dish ("saliva malodor"). Only in the case of saliva self-measurement was some objectivity observed. However, subjects tended to subsequently disregard the objective component in favor of their preconceived notions. Clinical studies have shown that objective improvements in malodor levels, following oral hygiene instruction and efficacious mouthrinsing, are not reflected in patients' self-reports (A. Fleming, unpublished data; Iwakura *et al.*, 1994).

In order to overcome the problems inherent in self-assessment and self-reporting, we have adopted the procedure of encouraging individuals to bring family members (or friends) to the consultations themselves. Accompanying persons are taught how to distinguish between oral and nasal malodor (Prinz, 1930). They are also asked to verify whether the individual's odor at the consultation resembles the odor which is usually present, both in terms of

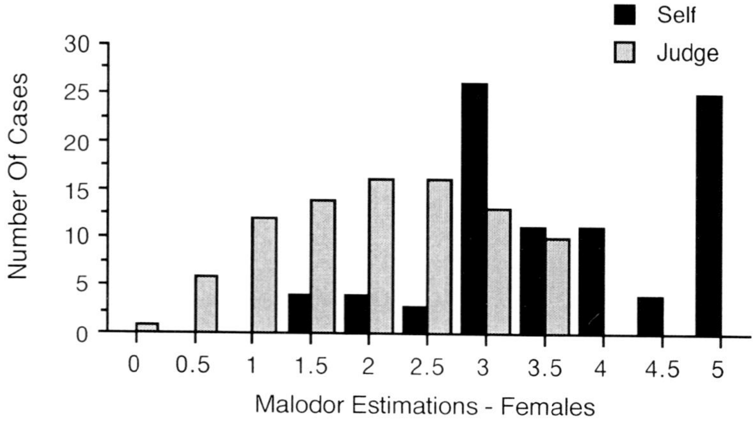

Figure 4. Odor judge whole mouth scores *vs.* self-estimation in females.

intensity and character. When a person presents at the clinic without appreciable odor, accompanying persons can provide crucial information on whether the problem actually exists at other times, or is imagined. Unfortunately, most of the individuals who grossly exaggerate the magnitude of their oral malodor are those who insist on arriving by themselves, and also tend to be most convinced that a problem does exist. The few individuals whom we have persuaded to seek psychological counsel have shown marked improvements (A. Goldstein, personal communication).

The underlying reasons leading people to believe, mistakenly, that they suffer from bad breath are not yet clear. Advertisements on the subject of bad breath may elicit unwarranted concerns in suggestible individuals. Others may notice a bad taste in their mouth and rightly or wrongly assume that it must be related to bad breath (see also Preti *et al.*, this volume). Foul-smelling calcified stones (tonsilloliths), coughed up from the tonsils, might also lead to assumptions of suffering from oral malodor (Pruet and Duplan, 1987). Some subjects recall having been told only once in their distant past that they had bad breath, yet continue to worry about it (see also Crohn and Drosd, 1942). In other cases, individuals cite a family member as having bad

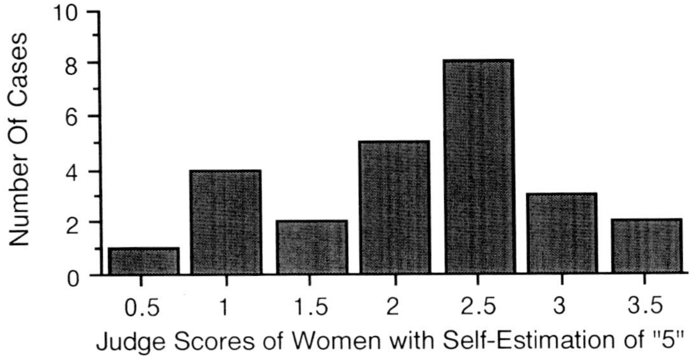

Figure 5. Distribution of whole mouth odor judge scores for women who gave self-estimates of "5".

breath; whether consciously or not, they may infer that they must suffer from the problem as well. For example, one woman in her early twenties came for consultation, accompanied by her father. The woman was convinced that she suffered from terrible malodor, and was, in the words of her father, "suicidal at times". Whereas the woman's breath was pristine, her father's was particularly offensive and could be smelled at a distance of well over a meter.

In some cases, imaginary bad breath is accompanied by clear pyschological pathologies (Davidson and Mukherjee, 1982; Hawkins, 1987; Iwu and Akpata, 1989). In some cases, the delusion of bad breath is so severe as to preclude classical psychotherapy. In one recent instance, a mother badgered her daughter repeatedly until the daughter was compelled to report falsely that her mother suffered from malodor. In another case, a local physician complained of suffering from oral malodor for over two decades and was extremely uncomfortable being near his patients. No objective basis for the complaint was found, and the physician was initially able to accept this finding on a professional level. Furthermore, after some brief coaxing, his spouse, with whom he had never spoken regarding this problem, arrived with him for consultation. She maintained that she had never noticed that her husband suffered from oral malodor (indeed, he professed to maintaining impeccable oral hygiene), and promised to tell him should he ever have bad breath in the future. However, despite the good initial prognosis, a telephone inquiry several months later revealed that the physician's concerns had not abated.

The above notwithstanding, it should be emphasized that the inability of odor judges, or even family members, to verify malodor in the reporting individual does not guarantee that subject's perceived bad breath is purely psychological in nature and devoid of any physiological basis or cue, such as persistent bad taste (Ayesh *et al.*, 1993; Preti *et al.*, this volume).

Although the major role of the posterior part of tongue dorsum as a source of oral malodor has been acknowledged for many years (Grapp, 1933), the underlying etiology has not yet been estabished. In earlier studies, we sampled this area using a gauze pad (Rosenberg *et al.*, 1991a). We subsequently found, however, that better results could be obtained by scraping the area with a plastic spoon. Furthermore, we observed that strong odors from this area are often accompanied by the presence of a yellowish discharge on the spoon, physically similar to postnasal drip. In the majority of cases, *i.e.*, the absence of a frank nasal infection, this secretion may be initially odorless, but following putrefaction on the tongue itself, a unique type of smell arises. For tongue brushing to be of value in oral malodor reduction, it must include removal of this coating from the tongue dorsum posterior, a difficult task due to gagging reflex.

Among some thousand subjects whom we have tested over the past years, there has not been a single instance in which the gastrointestinal tract appeared to be directly involved in oral malodor. This is in agreement with previous investigators who suggested that since the esophagus is collapsed, odors cannot escape except during belching (Prinz, 1930). Nevertheless, we continually encounter physicians and dentists who still believe bad breath to be primarily a gastrointestinal condition. Regrettably, many of our subjects had undergone gastroscopies before coming to see us.

Although the ear-nose-throat literature cites the tonsils as a not uncommon source of oral malodor (Costellani, 1936; Bogdasarian, 1986), this remains an open issue. Tonsils may harbor a variety of odorigenic microorganisms, including periodontal pathogens (van Winkelhoff *et al.*, 1986). Although we were able, in some cases, to detect foul odors on the tonsils themselves, as well as emanating from tonsilloliths (Pruet and Duplan, 1987), they did not necessarily lead to bad breath. Modern treatments, such as laser cryptolysis (Finkelstein, chapter 11, this volume), may help clarify the direct contribution of the tonsils to the oral halitus.

Summary

Among some three hundred individuals who sought advice regarding bad breath, over a eleven-month period in 1992, 60% were women. Whereas actual odor judge scores were higher for men, women gave higher self-estimation scores than men. Characteristic odor from the posterior tongue dorsum, sampled with a plastic spoon, was a major factor in the presenting malodor. We propose that this odor derives, mainly, from postnasal drip, which accumulates there. Although, in most cases, odors from the oral cavity itself were noted, malodor emanating from the nasal passages was not uncommon. In contrast, not one case of odor deriving from the stomach was found. Whereas, in many cases, the physiological causes of malodor can be rectified, the psychological element is relatively intractable. "Halitophobic" subjects with imaginary bad breath often remain convinced that they continue to have bad breath, despite professional counsel to the contrary. Finding ways to help the population of subjects with greatly overexaggerated concern of bad breath remains a challenge for future consideration.

Acknowledgments

We are grateful to Maayan Granot for her assistance in preparation and analysis of the data, and to I. Bar-Kokhav and Y. Finkelstein for helpful discussions.

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10

Non-Oral and Oral Aspects of Oral Malodor

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Oral malodor, halitosis, has been considered a worrisome problem for centuries. References to bad breath and remedies for this condition can be found in the writings of ancient Greece and Rome, including the works of Hippocrates, the "father" of medicine (460-377 BC; Geist, 1957). Several modern authors have suggested that bad breath may affect from 50-65% of the population at one time or another (Spouge, 1964).

Numerous reviews on oral malodor have pointed to a variety of factors in the oral cavity that can affect the intensity or degree of malodor produced (Tonzetich, 1977; Tonzetich, 1978; Kleinberg and Westbay, 1990). Tonzetich and colleagues are generally regarded as having defined the volatile sulfur compounds (VSC) mainly responsible for halitosis, their formation from non-volatile precursors and their patho-physiological effects on surrounding tissue (Tonzetich and Kestenbaum, 1969; Tonzetich, 1971; Tonzetich and

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Carpenter, 1971; Ng and Tonzetich, 1984). According to Tonzetich (1977), the VSC responsible for endogenous halitosis are hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethylsulfide (CH_3SCH_3).

The review by Attia and Marshall (1982) describes a host of systemic problems/pathologies that may contribute malodors to expired breath and mouth air. Unfortunately, few of these pathologies have been investigated via organoleptic evaluation and analytical instrumentation capable of confirming the molecular structures of the causative odors. Those pathologies that have been investigated using instrumentation are listed in Table 1, along with the compounds they contribute to the oral cavity and expired breath. In certain instances these systemically produced metabolites may also be perceived as a "bad taste" by the patient as will be discussed below in regard to the metabolic disorder, trimethylaminuria.

Our introduction into research involving oral odors and their use in diagnosis was via collaboration with Dr. Tonzetich. Initially, we focused upon oral VSC levels and how they varied during the menstrual cycle.

Numerous biochemical, cytological and microbial factors change in a cyclical fashion in concert with the changing levels of sex steroids during the menstrual cycle (Löe, 1965; Holm-Pedersen and Löe, 1967; Main and Ritchie, 1967; Lindhe *et al.*, 1969). Prior to our investigation, anecdotal reports in the literature suggested that some women had increased oral malodor during menses (Massler *et al.*, 1951). Results from our study suggest that levels of VSC in basal, morning mouth air tend to increase two- to four-fold around the day of ovulation (±48 h) and in the peri-menses days (Tonzetich *et al.*, 1978). Other smaller increases were seen in mid-follicular and mid-luteal phases. The sharp rise in VSC noted at mid-cycle corresponded closely to the ovulatory luteinizing hormone surge.

Our studies next examined the headspace above saliva. Studies by Tonzetich and coworkers (Tonzetich, 1971; Tonzetich and Carpenter, 1971; Tonzetich, 1977; Tonzetich *et al.*, 1978) had demonstrated that the headspace above incubated saliva from patients with periodontal disease was enriched in VSC. Consequently, we employed combined gas chromatography/mass spectrometry (GC/MS) to analyze the array of organic compounds, of varied structural types, in the headspace above saliva collected from normals and periodontally involved individuals. Applying high resolution gas chromatography with the mass spectrometer's inherent ability to yield structural

Table 1
Oral/Breath Volatiles Identified in Patients with Systemic Pathologies

Pathology	**References**
Diabetes mellitus: (ketonic breath) acetone and other ketones	Rooth and Ostenson, 1966
Uremia/kidney failure: (fishy odor) dimethylamine [$(CH_3)_2NH$; trimethylamine $(CH_3)_3N$]	Simenhoff *et al.*, 1977
Lung Carcinoma	
A. Acetone, methylethylketone and *n*-propanol	Gordon *et al.*, 1985
B. Aniline and *o*-toluidine	Preti *et al.*, 1988
Upper Respiratory/Oropharyngeal Carcinoma	
C_2-C_8, normal and branched organic acids	McGregor *et al.*, 1982; Preti *et al.*, 1992
Liver Disease	
A. Noncholestatic: H_2S, limonene	Friedman *et al.*, 1994
B. Primary biliary cirrhosis: H_2S	
C. Decompensated cirrhosis of the liver: (fetor hepaticus)	
C_2-C_5 aliphatic acids, methylmercaptan (CH_3SH), ethanethiol (CH_3CH_2SH) and dimethylsulfide (CH_3SCH_3)	Chen *et al.*, 1970a,b
Trimethylaminuria	Preti *et al.*, 1993
Trimethylamine	

Table 2
Pyridine and Methylpyridines from Headspace Above Saliva

	Pyridine	2-Picoline	3-/4-Picoline
Control (n=13)	5.2 ± 3.6[a]	ND[b]	ND
Patients (n=10)	328.6 ± 90.8	71.0 ± 28.5	296.3 ± 102.4

[a] mean ± SEM (ng/subject)
[b] ND = Not Detected

information and identity from nanograms of organic compounds showed distinct differences between the two groups as summarized in Table 2 (Kostelc *et al.,* 1981).

A variety of other compounds were also identified in the saliva headspace (Kostelc *et al.,* 1980), but only pyridine and picolines (methylpyridines) appeared to qualitatively differentiate controls from periodontally involved individuals. We further hypothesized that these aromatic amines might arise in active periodontal disease from the degradation of oral collagen. Pyridinoline is a cross-linking compound in oral collagen with a substituted pyridine structure, which could be metabolized by bacteria to the volatile pyridine and picolines we found; consequently, we felt that the presence of these compounds, in particular, the picolines, was indicative of active periodontal disease (Kostelc *et al.,* 1981).

As summarized above, our initial research into oral volatiles focused on oral volatile components that might be diagnostic of the patient's oral health rather than the relationship between oral malodor and oral health status. Because of our experience in the analysis of body and oral odors, however, dentists associated with the University of Pennsylvania Dental School, who also participated in private practices, began to refer patients who complained of persistent oral odor and/or bad taste. Many of these patients were found to have elevated levels of H_2S or CH_3SH (see Figure 1), although all appeared, by their dentists' assessments, to have good oral health. Consequently, except in several instances in which the patient's elevated VSC could be attributed

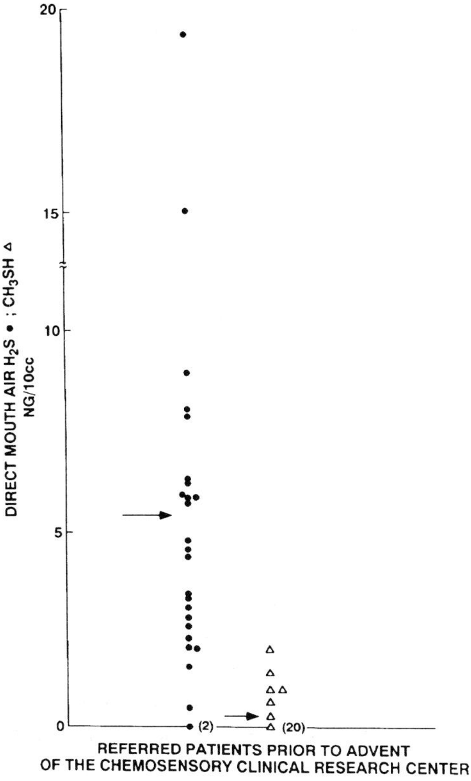

Figure 1. Levels of hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH) in the mouth air of patients referred to our laboratory prior to the establishment of our Chemosensory Clinical Research Center. Arrows indicate the mean levels of H_2S and CH_3SH. As noted on the graph, two patients had no measurable levels of H_2S while only six of the referred patients had non-zero levels of CH_3SH. These data suggest a very low production of CH_3SH in the mouth air of these patients, which is consistent with a good level of oral health.

to low salivary flow, no obvious cause for elevated VSC could be found. In addition, as can be seen in Figure 1, many patients did not have elevated VSC.

These types of patients became more frequent after creation of our

Chemosensory Clinical Research Center (CCRC), funded by a grant from the National Institute of Deafness and Other Communicative Disorders. Our CCRC is a collaborative effort between the Monell Chemical Senses Center and Thomas Jefferson Medical College. Four other CCRCs have been established throughout the United States; however, because of our analytical capabilities in the analysis of oral and other body odors, ours is the only one to accept referral of patients with odor production problems. Consequently, patients referred to us with dysgeusia, dysosmia and/or persistent oral malodor production are examined for the possible contribution of aberrations in oral/systemic chemistry.

We recently reported on data generated from patients seen in our CCRC during the first 3 years of operation (Preti *et al.*, 1992). Presented in that article was the protocol for analysis of all subjects by our Analytical Core Facility. The diagnostic protocol has been expanded for patients referred with long-term bad breath/bad taste; this is summarized in Table 3. The choline challenge test for trimethylaminuria (TMAU) developed by Tjoa and Fennessey (1991) has been included in our protocol to determine the prevalence of this metabolic disorder in our patient population and to aid in the development of a salivary assay for trimethylamine (TMA).

Trimethylaminuria

The odor-producing metabolic disease TMAU has recently been shown to present with symptoms of dysgeusia/dysosmia (Leopold *et al.*, 1990). Individuals whom we have seen with this disorder complain of a persistent, foul taste or odor that may be only temporarily relieved by normal hygienic procedures. Sufferers report that these symptoms are not only unpleasant but, since they seem so subjective, are often considered evidence of a psychiatric rather than a physiological disorder. Published studies support the fact that sufferers have no obvious physical or mental abnormality, may lead essentially normal lives, and practice good hygiene (Al-Waiz *et al.*, 1987; Leopold *et al.*, 1990; Tjoa and Fennessey, 1991). Many cases of TMAU go undiagnosed (albeit not undetected) due to the episodic nature of the primary symptom (excess production of trimethylamine, a volatile metabolite which has a foul, fishy flavor/odor) and ignorance of the disease and its etiology among many medical practitioners. One or both of these may lead to a diagnosis of

Table 3
Diagnostic Protocol for Referred Patients to the Analytical Core of the CCRC

1. FIRST MORNING VOIDED URINE (BASELINE FOR CHOLINE CHALLENGE TEST).
2. PATIENT PRESENTS IN FASTING CONDITION (NO COLOGNE, COSMETICS OR FRAGRANCES); QUESTIONNAIRE AND INTERVIEW.
3. ORGANOLEPTIC EVALUATION OF PATIENT'S BREATH: TWO JUDGES, SCALE OF 1-10.
4. ANALYSIS OF PATIENT'S MOUTH AIR BY GAS CHROMATOGRAPHY/FLAME PHOTOMETRIC DETECTED (GC/FPD) FOR VOLATILE SULFUR COMPOUNDS (VSC): H_2S, CH_3SH, $(CH_3)_2S$ (2-3 REPETITIONS).
5. DETERMINATION OF RESTING SALIVARY FLOW RATE.
6. ANALYSIS OF NASAL AIR FOR VSC LEVELS.
7. STIMULATED SALIVA ($\leq$ 10 ML) FOR INCUBATION/HEADSPACE ANALYSIS BY GC/FPD (VSC) AND GC/MS.
8. BASELINE SALIVA SAMPLE ($\leq$ 10 ML) FOR TRIMETHYLAMINE (TMA) ANALYSIS (ADJUST pH TO $\leq$ 3).
9. LUNG AIR COLLECTION (20 L) FOR ANALYSIS BY GC/FPD (VSC) AND GC/MS.
10. ADMINISTER 5 GRAMS OF CHOLINE FOR CHALLENGE TEST IN ca. 400 ML OF JUICE.
11. FOR THE NEXT 24 H PATIENT MUST
    A) COLLECT URINE EACH 8 H, FOR 24 H
    B) COLLECT 2 STIMULATED SALIVA SAMPLES ($\leq$ 10 ML) IN EACH 8 H TIME FRAME (ADJUST pH $\leq$ 3).
12. NEXT DAY
    A) PATIENT RETURNS ALL SAMPLES
    B) DISCUSSION OF RESULTS FROM DAY 1
    C) EXAMS BY
        1) CCRC DENTIST
        2) CCRC ENT (ODOR EVALUATION)
    D) SENSORY EXAM INCLUDING TMA THRESHOLD MEASUREMENT

poor hygiene or to the patient being referred to other specialists and often for psychiatric treatment.

The true prevalence of TMAU in the population is not known, but the prevalence of heterozygotes is estimated at about 1% among the Caucasian population in Britain (Al-Waiz *et al.*, 1987; Ayesh *et al.*, 1993). If this estimate is correct and the figure can be extrapolated to the US population as a whole, it would mean that over 2,500,000 individuals carry a copy of the defective gene and consequently 25,000 individuals are homozygous. It is important to note that our evidence (see below) suggests that heterozygotes as well as homozygotes may exhibit symptoms, such as dysgeusia/dysosmia. This figure may seem high given that only 27 were reported in the literature from 1970 to 1990 suggesting that TMAU is rare (Chen and Aiello, 1993); however, we have diagnosed 18 cases in our CCRC in a 4-year-period. This suggests that there may be a large, undiagnosed population yet to be revealed consistent with the relatively large incidence estimated in the British study.

We recently published the first report in the medical literature describing a TMAU sufferer presenting with the primary complaint of dysgeusia, which she described as a persistent bad taste. During the evaluation of this patient, it was found that she was actually complaining of a persistent bad odor (dysosmia), which she localized to her mouth and called a "taste" (Leopold *et al.*, 1990). Although dysosmia has not been generally noted in previous reports concerning TMAU patients, this symptom is part of the disease's presentation in the patients we have seen. The previous failure to identify dysosmia as a symptom of TMAU may be due to its subjective nature and to the fact that questions about persistent tastes or smells are not often posed during patient evaluations. In at least one other laboratory that investigates this disease, no chemosensory questions are raised (P.V. Fennessey, personal correspondence).

The precise biochemical nature of TMAU is not known. However, it is thought that it is a functional problem associated with metabolism of TMA to trimethylaminoxide (TMAO) in the liver. The best candidate for the catalysis of this reaction is the microsomal, flavin-containing monooxygenase (FMO). FMO is a catalytically diverse enzyme that can oxidize numerous drugs, pesticides, industrial chemicals and naturally-occurring plant toxins containing nitrogen, sulfur, selenium or phosphorous (Tynes *et al.*, 1986; Lawton and Philpot, 1993). FMO has also been shown to catalyze the metabolic N-oxidation of TMA to TMAO (Hlavica and Kehl, 1977).

This route of trimethylamine metabolism is subject to genetic polymorphism in humans (Al-Waiz *et al.*, 1987; 1988), resulting in individual differences in TMA, N-oxidation capacity. In addition, in at least one other mammal, the rat, there are also gender differences in FMO activities, with male rats exhibiting greater hepatic FMO catalyzed metabolism than females; this suggests a hormonal role in the regulation of xenobiotic metabolism by these enzymes (Lemoine *et al.*, 1991).

We believe that the presence of higher than normal levels of TMA in the saliva of TMAU patients may be responsible for the persistent bad taste/bad breath they report. Support for this contention is described below.

Patient Data

Our interest in TMAU and its relationship to our patient's presenting complaints of persistent, unpleasant odors and taste which may or may not be detected by other individuals, grew out of a patient referred several years ago. This patient has been described in detail previously (Leopold *et al.*, 1990; Preti *et al.*, 1992). Our initial report on this patient generated public interest in the popular press, resulting in a continuing stream of patients from all over the country. This experience prompted us to begin assessing TMA levels in all patients referred to us with long-term complaints of idiopathic oral malodor/bad taste.

The 52 patients are summarized in Table 4. These patients begin with our initial TMAU patient and end with patients seen at the end of 1992.

TMAU Positive Patients

Of the 52 patients who presented to us with persistent oral malodor/bad tastes, 18 (35%) were diagnosed with various forms of TMAU (Table 4). Women outnumbered men in this sample as a whole, but there was a significantly higher proportion of women among patients diagnosed as TMAU positive than among the remainder of our patients ($\chi^2 = 4.21$, $p<0.05$). Following the methods and reasoning of Al-Waiz *et al.* (1987; 1988) and Ayesh *et al.* (1993), we believe that patients #32 and #42 are likely homozygotes with the rest being heterozygotes. This distinction is based upon the percent of TMAO in urine after challenge with choline bitartrate, with proposed homozygotes showing ≤35% TMAO and heterozygotes having ≤85% TMAO.

Table 4
Diagnosis of 52 Patients

Case No.	Complaint	Age	Gender	Cause[a]
1	Persistent bad taste/oral malodor	36	F	TMAU; elevated salivary DMDS[b], indole, aniline
2	Longstanding fecal odor on breath	38	F	Oral anaerobes[c]
3	Foul odor on breath	29	F	lung air and salivary C_2-C_5 acids
4	Nasal malodor	33	M	Elevated VSC in lung and mouth air
5	Oral malodor	54	M	Cystathionuria[d]
6	Bad breath	33	F	Elevated mouth and lung air; H_2S
7	Bad breath	53	F	Elevated lung air H_2S
8	Persistent halitosis	38	F	ND[g]
9	Oral and body malodor	32	M	Elevated oral H_2S & CH_3SH; dental[e]
10	Malodor, nasal area	83	M	ND[g]
11	Oral malodor, metallic taste	29	F	Low salivary flow
12	Persistent oral malodor/bad taste	37	F	Elevated lung air; H_2S
13	Life-long oral malodor	62	M	TMAU
14	Persistent oral and body odor	46	M	ND[g]
15	Persistent oral malodor	57	F	Elevated mouth air CH_3SH
16	Persistent foul taste and smell	53	F	TMAU
17	Chronic halitosis	42	F	TMAU
18	Persistent body odor	56	F	ND[g]
19	Persistent oral/nasal odor	31	F	Elevated lung air, oral bitter taste and salivary H_2S
20	Fishy taste, bad smell	33	F	TMAU
21	Foul nasal odor	59	F	Elevated mouth air H_2S
22	Persistent oral malodor	43	F	TMAU; low salivary flow and bad taste
23	Chronic bad breath, foul taste	69	F	TMAU; elevated mouth air H_2S
24	Persistent oral malodor, dry mouth	39	F	Elevated lung and nasal air H_2S

Case No.	Complaint	Age	Gender	Cause[a]
25	Body, oral malodor	50	M	Low salivary flow; elevated saliva DMS; lung air DMDS and C_2-C_5 acids
26	Persistent foul odor/taste	43	M	Lung air C_2-C_5 acids and indole
27	Persistent oral malodor	41	F	TMAU; elevated, lung, mouth and nasal air H_2S
28	Persistent oral malodor	24	M	Elevated lung air VSC; C_2-C_5 acids
29	Persistent oral malodor	37	M	ND[g]
30	Persistent bad taste	51	F	ND[g]
31	Persistent bad taste and smell	45	F	TMAU; low salivary flow
32	Persistent oral malodor	42	F	TMAU
33	Persistent oral malodor	69	M	TMAU; low salivary flow
34	Persistent body odor	44	F	TMAU, low salivary flow; Elevated lung air C_2-C_5 acids
35	Persistent oral malodor	36	M	Low salivary flow
36	Persistent oral malodor/bad taste	45	F	TMAU; elevated salivary DMS; lung air C_2-C_5 acids
37	Persistent metallic taste	39	F	TMAU; elevated salivary DMS; malodor lung air C_2-C_5 acids
38	Metallic/bitter taste	40	F	Low salivary flow, high lung and mouth air H_2S
39	Persistent oral malodor/foul taste	29	M	Elevated mouth and lung air H_2S
40	Bad taste/oral malodor	12	F	Elevated mouth and lung air H_2S and salivary DMS
41	Persistent body odor	44	F	High VSC, lung, mouth, axillary odor
42	Persistent bad breath		F	TMAU; elevated lung air and mouth air H_2S
43	Bad taste and breath	73	F	Low salivary flow; elevated salivary; DMS
44	Bad breath/bad taste	49	F	ND[g]

Case No.	Complaint	Age	Gender	Cause[a]
45	Oral malodor/persistent taste	36	F	Low salivary flow; lung air C_2-C_5 acids; salivary DMS
46	Persistent bad breath	34	F	TMAU; elevated salivary VSC, DMS
47	Chronic and progressive halitosis	40	F	TMAU; lung air C_2-C_5 acids
48	Persistent bad taste/oral odor	44	M	Low salivary flow; elevated salivary DMS
49	Persistent body odor	43	F	ND[g]
50	Persistent bad breath	43	M	Mouth air CH_3SH, hiatal hernia, lung air, salivary C_2-C_6 acids; elevated salivary DMDS; DMS[f]
51	Chronic halitosis	32	F	TMAU; elevated lung air DMS, DMDS, salivary C_2-C_4 acids
52	Persistent bad breath	35	F	Mouth air CH_3SH, lung air DMS

[a] "Elevated" in this Table refers to a volatile compound being > the mean value found in normal controls + twice the standard deviation.

[b] Abbreviations: DMDS - dimethyldisulfide; C_2-C_5 acids = acetic, propionic, isobutyric, butyric and isovaleric acids; H_2S = hydrogen sulfide; CH_3SH = methyl mercaptan; DMS = dimethylsulfide.

[c] Anaerobic cultures taken in several areas of this patient's oral cavity revealed anaerobic pathogens associated with localized, active periodontal disease.

[d] Duplicate, 24 hr urinary amino acid analysis, revealed higher-than-normal levels of cystathionine, suggesting the presence of cystathionuria.

[e] This patient was referred by his family doctor but admitted to not having been to the dentist for several years. After oral prophylaxis, his oral symptoms were ameliorated.

[f] Anaerobic cultures of tongue posterior suggest colonization by odor producing organisms.

[g] Not determined.

The average age of TMAU affected patients was 44.42 ± 16.25 (SD) with a range of 32-69 years. The remaining patients in Table 4 had an average age of 41.47 ± 15.70 (SD) with a range of 12-73 years. Although several patients affected by TMAU had levels of other volatile organic compounds above those found in normal controls, no consistent pattern was seen.

We have recently reported on the results from initial analyses of saliva for TMA using an assay developed in our lab (Preti *et al.*, 1993). This assay utilizes deuterium-labeled trimethylamine as an internal standard and gas chromatography/mass spectrometry for separation, identification and quantitation of both endogenous TMA and the deuterated internal standard. Inter-injection variation was determined using various concentrations of TMA•HCl and deuterated TMA•HCl during generation of the standard curve.

Although TMA levels have been examined in a number of body fluids and tissue samples, salivary levels of this compound have not been reported. Although simple in structure, the compound is a gas at room temperature and extremely polar. These factors make TMA potentially difficult to analyze at the low levels likely to be seen in saliva. However, we have been able to obtain reliable data on salivary levels of this compound. The preliminary results shown in Table 5 suggest TMA is present at higher levels in TMAU patients than in controls. As expected salivary levels in both patients and controls are much lower than those reported in human urine and blood (Tjoa and Fennessey, 1991).

Non-TMAU Patients

Among the 52 patients, 34 patients presenting with idiopathic complaints of persistent bad taste/smell were found to be negative for TMAU. However, as can be seen in Table 6, these patients did appear to have an elevation of VSC relative to normal controls which may contribute to their complaints. As discussed above, production of these compounds may be attributed to poor oral health. However, evaluation of our patients by one of us (RF), as well as by referring dentists, demonstrated most patients to have generally healthy teeth and periodontal tissue; only one (patient #9) was found to have an obvious oral health problem. Anaerobic cultures of saliva, tooth and/or tongue plaque from patients #2, 45, 48 and 50 also found that each patient had one or more VSC-producing organism (Tonzetich and McBride, 1981; Kleinberg and Westbay, 1990).

Table 5
Baseline (Precholine) Salivary TMA Levels

SAMPLE (nm/ml)[a]		**TMA (ng/ml ± SD)**
Control 1	Male; age 48 yrs	130.13 ± 9.37
Control 2	Male; age 31 yrs	146.75 ± 20.55
Patient #35[b]	TMAU Negative (male)	162.20 ± 1.70
Patient #42[b]	TMAU Positive (female)	605.40 ± 47.22

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[a] Levels seen in human blood and urine are reported to be 40 nm/g and 30-70 µm/h (Tjoa and Fennessey, 1991), respectively (urinary levels vary with technique and laboratory).

[b] See Table 4

Figures 2 and 3 show the mouth and lung air levels of H_2S in the normal controls and patients, respectively. As may be seen in these figures there is a group of idiopathic malodor producers which have higher H_2S levels than all other patients and controls.

The levels of H_2S and CH_3SH in the mouth air of all individual patients and normal controls are plotted in Figure 4. As seen in Figure 4, only one TMAU-positive patient had measurable levels of mouth air CH_3SH, and seven of the idiopathic oral malodor producers (non-TMAU patients) had CH_3SH. High levels of CH_3SH in conjunction with an elevated ratio of CH_3SH/H_2S have been associated with periodontal involvement by Yaegaki and Sanada (1992a,b). These researchers report mean levels of CH_3SH in the mouth air of periodontally involved patients to be 44.0 ng/10 ml (vs 2.6 ng/10 ml for controls). The mean CH_3SH/H_2S ratio in the periodontally involved patients of Yaegaki and Sanada (1992a) was 4.64. Consequently the low CH_3SH levels in our patient group attest to their good periodontal health, as confirmed by oral examinations. Table 7 compares the data of Yaegaki and Sanada (1992a,b) with those of our idiopathic malodor producers.

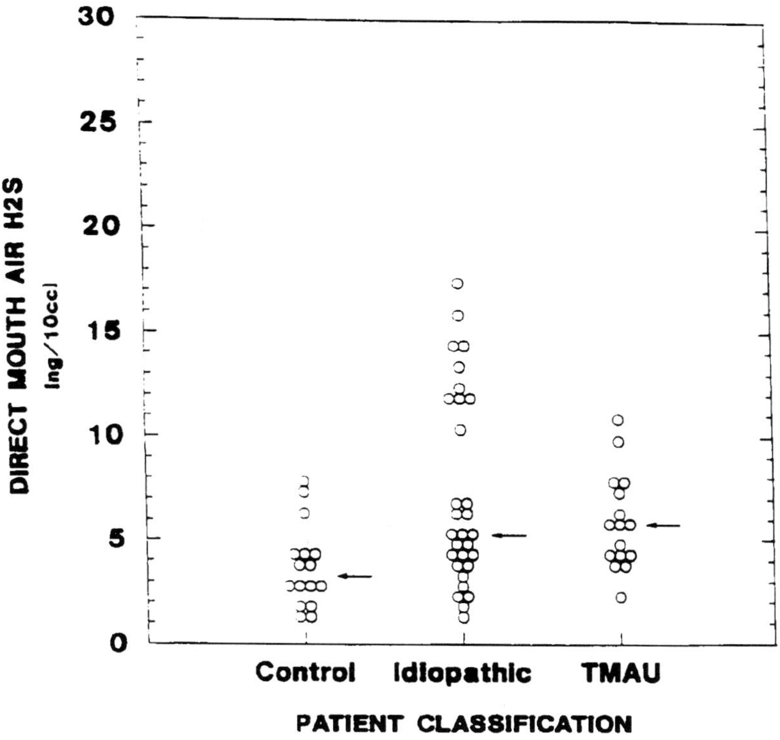

Figure 2. Levels of mouth air H_2S in patients and normal controls who have been tested by the Analytical Core of our Chemosensory Clinical Research Center. Idiopathic malodor producers and patients suffering from trimethylaminuria (TMAU) are those listed in Table 4 of the text. Arrows indicate the median levels of H_2S in each of the three groups.

The possible role of C_2-C_5 aliphatic acids in the lung air of our referred patients can only be inferred from the data presented here. Only one of our controls showed detectable levels of acetic acid in 20 liters of lung air; however, as seen in Table 4, a number of our referred patients had detectable levels of each of these acids. As noted in our earlier report (Preti *et al.*, 1992), volatile C_2-C_5 acids in the lung air of patients was one of the groups of compounds that distinguished them from controls.

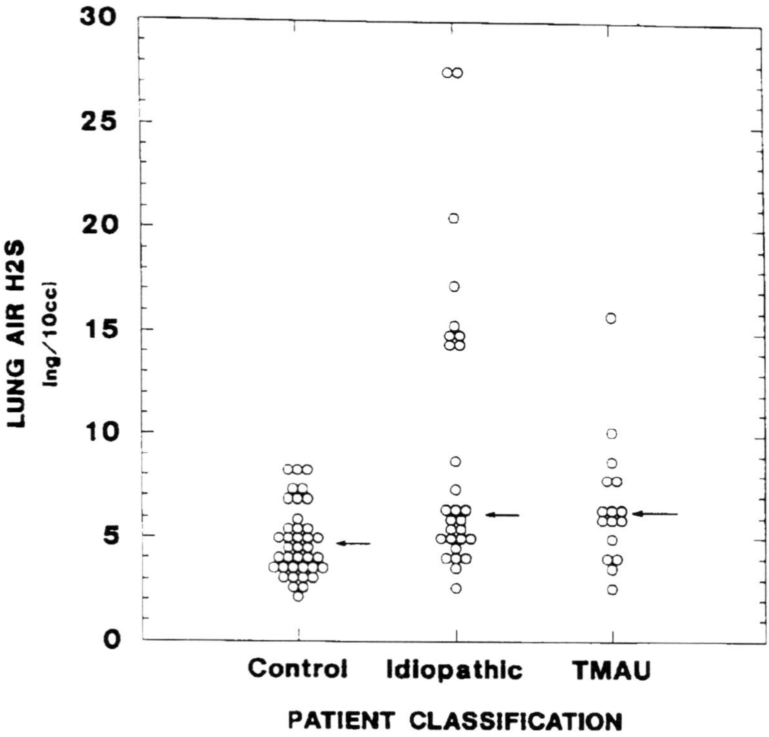

Figure 3. Lung air H_2S levels measured in normal controls as well as patients with idiopathic malodor production and TMAU that are listed in Table 4. Arrows indicate the median levels of H_2S found in each of the groups.

Sensory Testing

As part of our evaluation, all patients were given an examination by the CCRC dentist and otolaryngologist to determine if obvious head/neck or oral problems contributed to their persistent oral symptoms. In addition, all were given a standard battery of tests for olfactory and gustatory dysfunction (Cowart, 1989; Cowart *et al.*, 1993). The sensory evaluation of

Table 6
Mouth and Lung Air Levels of H_2S (ng/10cc)[a]

	N (Mouth)	VSC (Mouth)	N (Lung)	VSC (Lung)[b]
Normal Controls	16	3.62 ± 2.0	39	4.75 ± 1.66
TMAU Patients	18	5.99 ± 2.66	18	6.85 ± 3.84
Idiopathic Oral Malodor	34	6.55 ± 4.72	31	8.56 ± 6.82

[a] The three groups differed significantly in the amount of mouth and lung VSC ($p<0.04$ in for both mouth and lung, ANOVA); note: lung air measures of VSC were performed on a group of 39 normal controls whose mean age was 57.8 ± 32.5 yr.

[b] This group included the 16 controls used for mouth air VSC measures. Those patients with idiopathic malodor production (non-TMAU affected) produced more H_2S in lung air ($p < 0.003$; Tukey-Kramer) and mouth air ($p < 0.03$; Tukey-Kramer) VSC than the normal controls. No other individual comparisons were statistically significant.

malodor patients referred to the CCRC is designed to measure whether normal olfactory and gustatory abilities are present. The ability to identify odors and tastes is assessed, as well as absolute olfactory sensitivity (detection threshold) to two odor compounds, phenylethyl alcohol (PEA) and pyridine (PYR). We have also recently begun measuring olfactory sensitivity to trimethylamine in order to assess the feasibility of using a chemosensory test as a clinical screening tool for TMAU.

Among the 18 patients diagnosed with TMAU listed in Table 4, 16 underwent sensory testing. When compared with the normal controls tested in our CCRC, the mean olfactory thresholds for PEA and PYR of both TMAU and non-TMAU sufferers (idiopathic malodor patients) were not significantly different. Further, all TMAU patients tested within the normal range in their odor and taste identification tests. TMAU patients are

Table 7
Mean Levels of CH_3SH and CH_3SH/H_2S in Mouth Air

	CH_3SH (ng/10cc)	CH_3SH/H_2S
Idiopathic malodor patients[a] (n=7)	5.20 ± 3.03	1.17 ± 1.19
Periodontally involved patients[b] (n=17)	44.0	4.64
Controls[1] (n=14)	2.6	0.58

[a] From Table 4: includes only those patients with mouth air levels of CH_3SH shown in Figure 4.

[b] From Yaegaki and Sanada, 1992a.

hypothesized to have a deficit in TMA perception, possibly due to higher endogenous TMA levels. This is being further explored using a TMA dilution series to measure olfactory thresholds for this compound (as noted above).

Discussion of Patient Data

As mentioned above, only 27 cases of TMAU were reported in the literature prior to 1990 (Chen and Aiello, 1993); consequently, our 18 diagnosed cases represent the largest number of cases seen by one laboratory. Prior reports of TMAU cases also suggest female predominance (16 females vs. 11 males; Chen and Aiello, 1993) in patients suffering from this disorder which is consistent with our findings (16 of our 18 TMAU patients were female). However, the paucity of data regarding the true etiology of this disorder does not permit us to determine why women may be more frequently affected or may seek help more often than affected males.

Our studies also suggest that patients suffering from TMAU do not appear to have elevated VSC levels, although some individual exceptions to this are seen in Table 4 and in Figure 4. In light of this it should be noted that sulfur-containing drugs, pesticides and amino acids are converted to their S-oxides by hepatic FMOs (Zeigler, 1988). Consequently, VSC formed in the gut by degradation of dietary components should also be subject to oxidation by FMO. Although it has not been examined in TMAU-affected

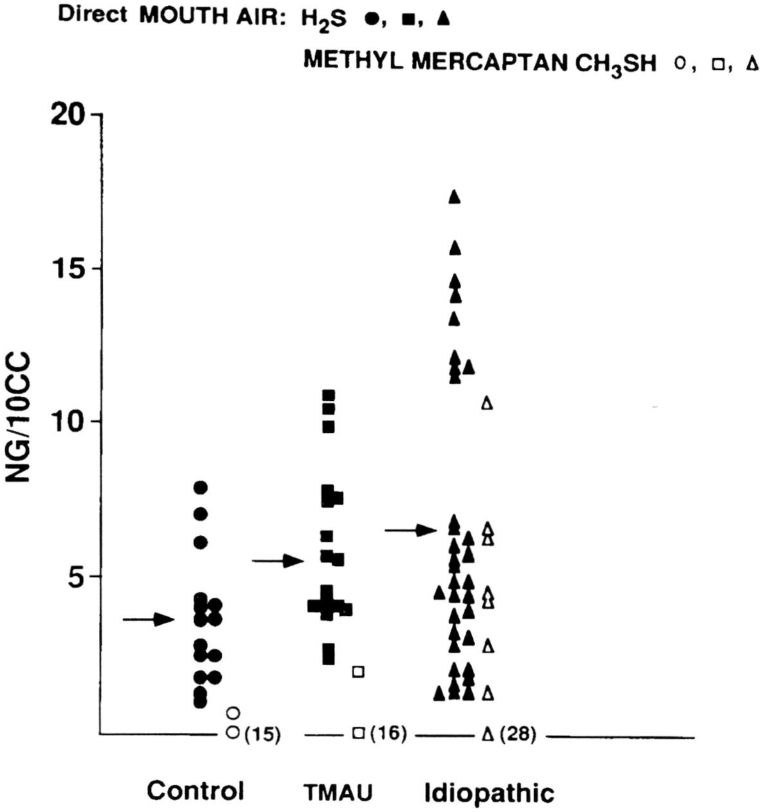

Figure 4. Mouth air levels of H_2S and CH_3SH found in normal controls as well as idiopathic malodor producers and patients suffering from TMAU. The latter two groups are listed in Table 4. For each of the three groups all H_2S levels were non-zero; however, only one normal control and one TMAU patient had values of CH_3SH greater than zero whereas there were seven idiopathic malodor producers that had greater than zero values. Arrows indicate the mean level of H_2S found in the mouth air of each of the patient groups.

patients (no clinical challenge test for S-oxidation capacity is available), some of these individuals might possess higher-than-normal levels of VSC if their diet contained sufficient S-containing precursors. Elevated VSC levels do not, however, appear to be primarily responsible for the oral malodor/bad taste that TMAU patients report, although they may be in non-TMAU patients with these symptoms.

If our hypothesis is correct and oral TMA levels are the source of the oral complaints in TMAU patients, then reduction of TMA will ultimately be the most complete solution to their problem. However, since this is a genetic disorder, no cure is yet available. Limiting intake of choline, the precursor for TMA, is not easily accomplished, due to the widespread presence of this substance in a variety of foods. Although other treatments have been suggested in the literature (Pike *et al.,* 1988), our clinical experience suggests that none offer anything more than temporary relief.

As a group, the non-TMAU patients have significantly greater levels of both mouth air and lung air H_2S. Whereas mouth air VSC have been shown to be generated by bacterial metabolism in the oral cavity, lung air sulfur compounds and volatile aliphatic acids are thought to arise from bacterial processes within the gut or upper air passages (Chen *et al.*, 1970a,b). Although acetic and propionic acids have been concentrated from large volumes (≥100 liters) of lung air of healthy individuals (Chen *et al.*, 1970a), large amounts of these compounds (≥4.0 µg/20 liters of total C_2-C_5 acids) have only been documented in the lung air of patients with decompensated cirrhosis of the liver (Chen *et al.*, 1970a,b) and carcinoma of the larynx (McGregor *et al.*, 1982; Preti *et al.*, 1992). Since the lung air of a number of our patients contained C_2-C_5 acid in the concentration range of 20-100 ng/liter, it is quite possible that these compounds may contribute to their persistent dysgeusia/dysosmia. The olfactory detection thresholds for these compounds is in the range of 1-7 ppb in air, which is well within the concentration ranges we have found. Consequently, while our patients with idiopathic malodor/bad taste complaints (non-TMAU affected) do not appear to have serious disorders (*i.e.*, cirrhosis or cancer), our data suggest the possibility of an undiagnosed problem in these patients.

Data presented at the First International Workshop on Oral Malodor (see McCulloch and Bosy, this volume) as well as by Bosy *et al.* (1994) also suggest that both the posterior, dorsal surface of the tongue and soft palate are often ignored areas where volatile, odoriferous, organic compounds may arise by anaerobic bacterial growth. Whether our patients who are currently classified as "idiopathic" have some manifestation of this anaerobic infection is currently being explored.

Current Trends in Oral Malodor Research

The results presented at the First International Workshop on Oral Malodor, in conjunction with the availability of a low-cost, easy-to-use, albeit non-specific, detector of VSC ("Halimeter") strongly suggest that interest among oral health/dental professionals in bad breath will continue to rise.

A particularly interesting result discussed at this conference has been the re-awakening of interest and focus on the posterior dorsal surface of the tongue as a site for malodor production (Grapp, 1933). Reviews on oral malodor published prior to 1970 (*e.g.*, Spouge, 1964), as well as a number of papers by Tonzetich and coworkers (Tonzetich and Ng, 1976; Tonzetich, 1977; 1978) that examined reduction of oral VSC by various dental hygiene procedures, strongly suggest that keeping the tongue clean is of major importance in reduction of oral malodor. The tongue provides an excellent harboring place for anaerobic organisms to proliferate (even in a patient with "good oral health") and to create malodors and bad tastes for the patient.

In addition to the Halimeter, other "chair side" diagnostic tools that may promote a more facile diagnosis of anaerobic, odor-producing growth in the mouth are the BANA test (Loesche *et al.*, 1990; De Boever *et al.*, 1994; Kozlovsky *et al.*, 1994) and colorimetric indicators that would demonstrate VSC production in gingival and/or tongue plaque. These methods are neither as specific nor likely to be as quantitative as the gas chromatographic methods developed by Tonzetich and coworkers (see Tonzetich, 1977 for review). The latter have traditionally been used by researchers in both academic and industrial laboratories. In spite of the ease with which these GC techniques can be used, the gas chromatograph with a flame photometric detector has not become a fixture at the dental office, clinic or dental school. Consequently, the Halimeter may help introduce some semblance of analytical instrumentation capable of giving an objective measure of a subject's oral malodor to the practicing clinician.

Several methods for treatment of the anaerobic tongue growth have been discussed proposed: a two-phase oil-water mouthwash, a highly antimicrobial (chlorhexidene) mouthwash, and tongue cleaning (debridement). Data on malodor reduction from these procedures have been published (Bosy *et al.*, 1994; Rosenberg *et al.*, 1991; 1992; Tonzetich, 1978; Yaegaki and Sanada, 1992b). Each appear to reduce VSC, but it remains to be seen which has the best long-term efficacy with minimal side effects.

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point of view of an otolaryngologist, with particular emphasis on modern equipment for diagnosis and therapeutic approaches.

Chronic Sinusitis as a Cause of Halitosis

It is estimated that tens of millions of Americans have sinusitis (Moss and Parsons, 1985; National Health Survey, 1986). Some 16 million Americans with sinusitis were referred to physicians in 1989 alone (National Disease and Therapeutic Index, 1988-89). Upper tract chronic and occult infections are usually located in the paranasal sinuses (Finkelstein *et al.*, 1989; Finkelstein *et al.*, 1994). Is chronic sinusitis a common disease which may occasionally cause halitosis? To discuss this topic, the diagnostic approach to sinusitis must be elucidated.

The sinuses are air-containing spaces within the confines of the bony facial skeleton. The sinuses are drained into the nasal cavity by their ostia. Continuous ventilation from the nasal cavity to the sinuses and mucociliary clearance from the sinuses to the nose are essential for proper function. Normally the sinuses are not sterile. Microorganisms penetrate with the air but the normal mucociliary clearance impedes them from significant pathologic proliferation within the sinuses (Brook, 1981). Efficient ventilation and mucociliary clearance of the large sinuses require patent ostia. The osteomeatal complex includes the part of the ethmoidal labyrinth that surrounds the ostia of the maxillary and frontal sinuses. Therefore, malformation and diseases of the osteomeatal complex may occlude the ostia of the large sinuses resulting in stasis and subsequent infection in the large sinuses (Stammberger, 1986a,b). Chronic ethmoiditis is a very common disease, and when present exclusively, is usually undiagnosed, particularly when modern approaches are not applied. Chronic sinusitis is substantially more difficult to recognize than acute sinusitis. The clinical presentation of chronic sinusitis often mimics that of other conditions. In many patients the primary presenting complaints are exclusively related to postnasal drip, such as cough, mildly productive cleansing of the throat, coated tongue or halitosis. A more thorough history may reveal nasal obstruction, congestion or postnasal drip. The old and routinely conducted anterior rhinoscopic examination (Figure 1), as well as the plain sinus radiographs, are of very limited value. Therefore, in the past, many patients have been defined as chronic complainers because their anterior rhinoscopic examination and their plain sinus radio-

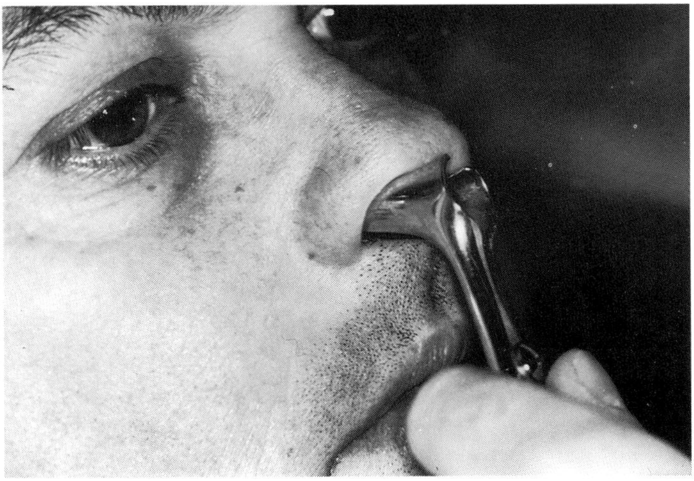

Figure 1. Anterior rhinoscopy performed by nasal speculum.

graphs were normal. The modern flexible and rigid endoscopes allow the clinician, in the office setting, to systematically examine the areas of inflammation or pathologic drainage in the intranasal structures. For the routine clinical examination I prefer to use the flexible endoscope because it allows penetration into narrow intranasal passages which are not accessible by the rigid telescopes. The flexible endoscope is more comfortable for the adult patient and allows examination of children. In addition, the flexible endoscope allows a complete examination not only of the nose but also of the naso-oro-hypopharynx and larynx. Observing the hypopharynx from above with the nasopharyngoscope, the base of tongue, as well as the lingual tonsil, are evident. In many patients with sinusitis and halitosis, the base of the tongue is coated. Thus, it may be hypothesized that the postnasal drip of purulent material continuously drops onto the base of tongue where a second seat of chronic infection is formed and the microbiota change accordingly; this may be responsible for the halitosis in these patients (Rosenberg and Leib, this volume).

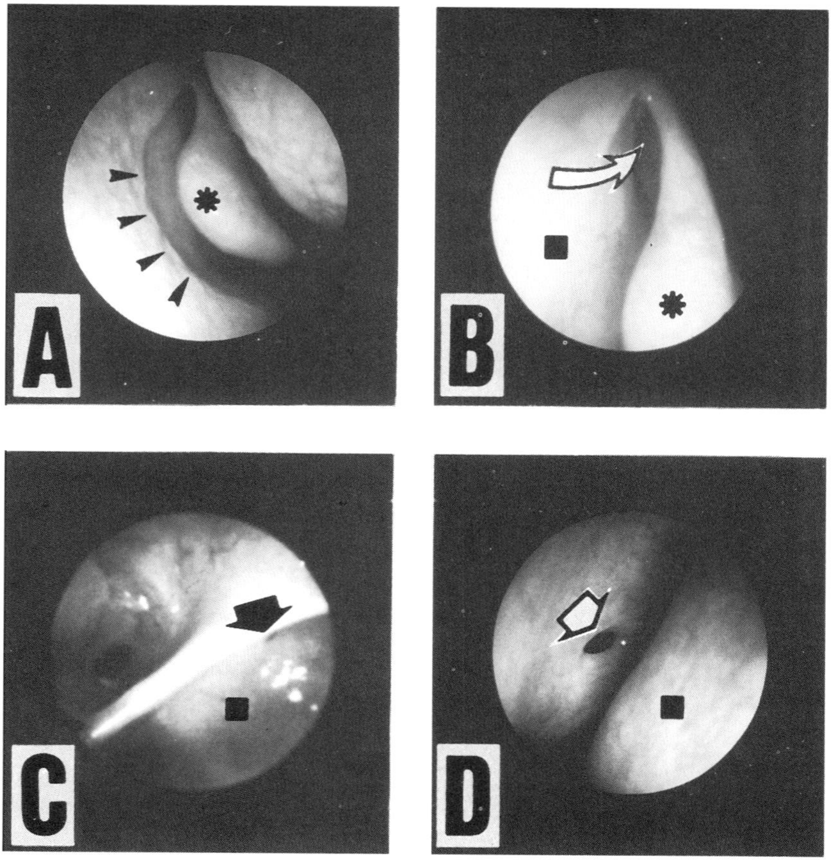

Figure 2. Endoscopic view of normal intranasal structures. A) normal right middle turbinate (asterisk). The arrowheads on the uncinate process indicate the middle meatus. B) Right frontal recess (empty curved arrow) where the frontal sinus drains between the lateral nasal wall (black square) and middle turbinate (asterisk). C) Left maxillary ostium (black arrow) through which normal maxillary mucosa (black square) is seen. D) Left accessory maxillary ostium (white empty arrow). The lateral aspect of middle turbinate is indicated by a black square.

Both endoscopy and computerized tomography (CT) have completely changed the methods of diagnosis and treatment of sinusitis. Nasal endoscopy provides essential qualitative information and allows one to evaluate the

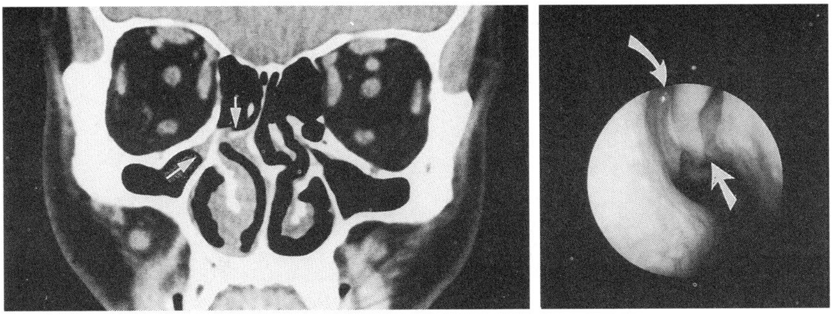

Figure 3. Deviated nasal septum and prominent vomerine bony spur which penetrate the middle meatus and occlude the maxillary ostium. Left: CT scan coronal section. Vertical arrow = spur, left oblique arrow = occluded maxillary vestibulum. Right: endoscopic view. The inferior arrow indicates the vomerine spur. The superior curved arrow inidcates the edematous tissue in the middle meatus.

appearance of the mucosa, to diagnose polyposis or polypoid disease and to evaluate the nature of secretions (*e.g.*, watery or thick mucous, purulent white, yellowish or green). A complete view of the intranasal three-dimensional structures and interrelationships between them is afforded (Figure 2). Anatomic abnormalities, such as vomerine spur (Figure 3), paradoxical curvature (paradoxically bent concha media) and expansion of the middle turbinate (concha bullosa, Figure 4, left) which may occlude sinusal ostia, are diagnosed. CT scanning provides information regarding the three-dimensional structure of the paranasal sinuses complex (Figure 4, left and Figure 5, left). However, endoscopy provides additional useful information regarding small areas of pathologic involvement which may not be revealed by computerized tomography. When purulent discharge is found endoscopically, sinusitis is diagnosed even in the presence of a normal radiographic examination. Whenever sinusitis is diagnosed, its cause should be determined. The underlying factor could be an occasional upper respiratory tract infection, malformed intra-nasal structures, chronic mucosal disease or, in rare cases, a primary defect of mucociliary clearance (immotile cilia syndrome).

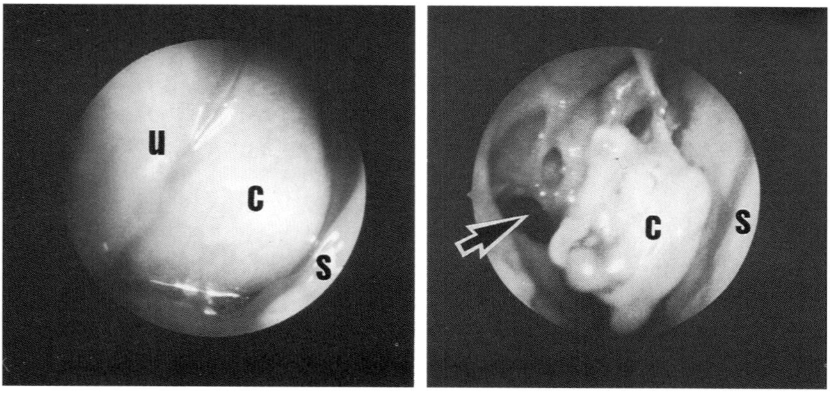

Figure 4. Endoscopic view of right concha bullosa. Left: preopertive view. Right: postoperative view: the maxillary ostium is wide open (arrow). Above the ostium open ethmoidal cells are: c: concha bullosa; s: nasal septum; u; uncinate process (lateral nasal wall).

A typical case illustrating the above is presented in Figures 4 and 5. A 55-year-old woman had a 5-year history of malodor and postnasal drip. Previous examinations by general practitioners, otolaryngologists, pulmonologists and allergologists were all negative and she was referred to a psychiatrist. On endoscopic examination of the right nasal cavity, a large concha bullosa was found (Figure 4, left). The anterior middle meatus was completely obstructed and thus it was impossible to penetrate with the endoscope and to examine the ostia of the sinuses. A small quantity of purulent discharge was found on the posterior nasal floor and it was impossible to pinpoint its origin. CT showed that the middle turbinate contained a large ethmoidal cell (Figure 5, left). Diseased concha bullosa may represent a localized ethmoiditis and thus form a unique seat of infection which is not observed by anterior rhinoscopy or plain radiographs. Concha bullosa may also obstruct the adjacent maxillary ostia causing maxillary sinusitis. Thus the source of the purulent discharge may be within the turbinate or the maxillary sinus. If correct diagnosis of the underlying factor of the maxillary sinusitis is not made, treatment may be limited to the secondary maxillary sinusitis, and the patient may continue to suffer from postnasal drip and bad breath.

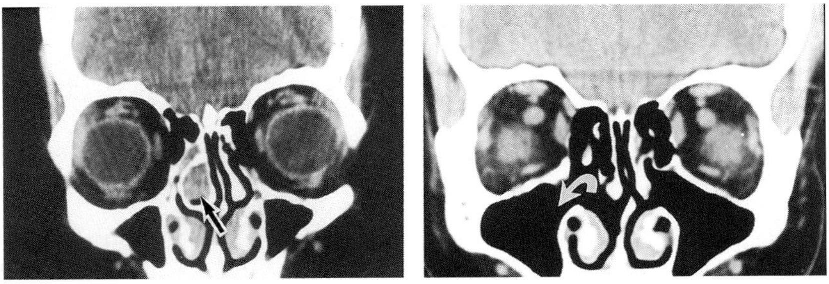

Figure 5. Coronal computized tomography section of the large concha bullosa shown in Figure 4. Left : preopertive view. Right: postoperative view.

The microbiology of chronic sinusitis is different than that of acute sinusitis. In acute disease, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae* are the main microorganisms (Brook, 1981). In chronic sinusitis the most common organisms are anaerobic gram-positive cocci, such as staphylococci and streptococci (Brook, 1981; Wald, 1992). In addition, *Bacteroides* species and *Fusobacteria* species are also recovered (Brook, 1981; Wald, 1992). These may include species which are capable of producing volatile sulfur-containing compounds (Tonzetich and McBride, 1981; Claesson *et al.*, 1990). Bad breath is worse when the origin of the sinusitis is odontogenic. Postnasal drip is a common finding in patients presenting with halitosis and coated tongue. Postnasal drip may not be revealed, even in a meticulous peroral and anterior rhinoscopic examination; thus, endoscopy is required for successful diagnosis. Nasal malformations and diseases may cause nasal obstruction. Sinusitis may cause congestion of inferior nasal turbinates and thus nasal obstruction. The surgical management decision regarding nasal obstruction relies on endoscopy and, if necessary, CT scanning. Nasal breathing is also important for adequate continuous clearance of saliva. Therefore, oral breathers may have bad breath. Nasal obstruction may cause snoring. Heavy snorers may also suffer from dry mouth in the morning and concomitant bad breath. Although elimination of nasal obstruction is important, it is useful in preventing nocturnal oral breathing and snoring in only a fraction of the adult snorers. Patients with halitosis in whom chronic nasal and paranasal sinuses disease is diagnosed need adequate treatment.

Prolonged antibiotic treatments of chronic sinusitis are not only useless, but may raise bacterial resistance and may lead to fungal sinusitis. Chronic sinusitis that fails to respond to rational medical treatment is usually performed by endoscopic endonasal surgery (FESS) (Stammberger, 1986; Lazar *et al.*, 1993; Matthews *et al.*, 1991). The operation is performed intranasally under local anesthesia and requires hospitalization of up to a day, if at all. The current surgical management is aimed at eliminating the primary pathologic area and thus restoring sinus ventilation and mucociliary clearance. Anatomic abnormalities are corrected and diseased tissue from the osteomeatal complex is removed.

The postoperative endoscopic and CT results are shown in Figure 4 (right) and Figure 5 (right), respectively. The medial wall of the concha bullosa was excised and the maxillary ostia opened. There is no purulent secretion and the mucosa is of normal appearance. The endoscopic surgery is effective for extensive polypoid disease of the sinuses as well.

In patients with halitosis, localization of the of primary infection is needed. Whereas the clinical diagnosis is easily made even at a distance, we are often unable to define the site of the infection. At present, clinical diagnosis and management of halitosis are commonly guided primarily by intuition, imagination and anecdotes. Otolaryngologic examination, including endoscopy, improves our understanding of the mechanisms of halitosis and makes a contribution toward diagnostic profiles, differentiating halitosis of different origins. However, patients with chronic sinusitis may have other underlying causes of halitosis. Both qualitative and qualitative data could be of value for topodiagnosis of malodor. Thus, there is also a need for development of site-specific sampling methods within the upper respiratory tract. Quantitative data on malodor production at various sites might provide information on metabolism at specific sites (Rosenberg and McCulloch, 1992).

Chronic Tonsillitis as a Cause of Halitosis

The palatine tonsils are localized, immunologically active lymphoid tissue, which are part of the lymphoid system. They contain various types of lymphocytes and secrete antibodies. The tonsillar crypts are twisted tubular invaginations extending from the tonsillar surface deeply through the parenchyma. Exfoliated epithelium, keratin debris and foreign particles may be retained in the crypts.

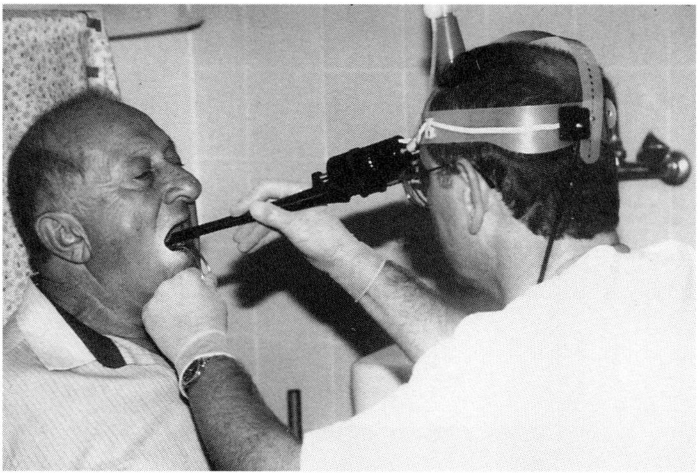

Figure 6. Laser cryptolysis in the clinic with Sharplan CO_2 Laser.

A great deal of information is available on the role of the tonsils in infectious disease, snoring and obstructive sleep apnea. However, chronic tonsillitis with tonsillolith formation may also be a cause of halitosis. Caseous tonsillitis is a variant of chronic tonsillitis. In these tonsils caseous secretion (debris) is retained in the tonsillar crypts forming calcareous concretions. The tonsilloliths or tonsillar calculi are usually multiple friable concretions but in some patients a single wide stone which distends the crypt may be found. Apparent halitosis is the most common symptom of this entity. Some patients complain about periodic halitosis and some of these patients complain that calculi which have a fetid smell are periodically produced. However, in others the disease is asymptomatic. In the author's experience, halitosis of tonsillar origin is much more common in patients with chronic tonsillitis, but without debris retention and tonsillolith formation. These patients may report that when they squeeze their tonsils, a strong foul odor is emitted. Many of them squeeze the tonsils every morning thus cleaning them temporarily from putrid odor discharge. On examination, the tonsils may or may not be hypertrophied, are usually not hyperemic and often have an innocent appearance.

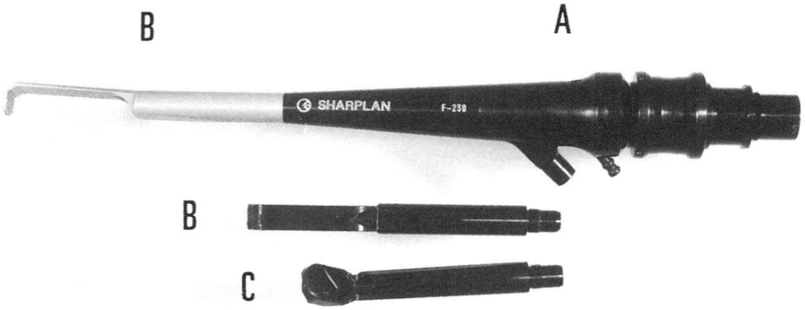

Figure 7. Sharplan® pharyngeal defocusing handpiece (main handpiece unit), and a reflective angled mirror tip. A: the main handpiece unit; B: backstop extender. C: a reflective angled mirror tip.

In routine examinations, following washing the glove and smelling it (control), I squeeze the tonsils and smell the odor on the gloves. In many cases the diagnosis can be made based on the fetid mucoid discharge. If possible, a family member is consulted to determine whether the smell on the glove approximates the patient's typical oral malodor.

It is of interest that mixed aerobic and anaerobic microbiota are present in the core of "normal" tonsils, without history of recurrent tonsillitis (Brook and Foote, 1990). Even in "normal" uninflamed tonsils bacteria are present (Brook and Foote, 1990), including anaerobes such as *Fusobacterium* and *Bacteroides* species which are capable of producing volatile sulfur-containing compounds (Tonzetich and McBride, 1981; Claesson *et al.*, 1990).

Antibiotic treatment with metronidazole may be administered to treat tonsil malodor. In most cases the results are initially satisfactory and the odor disappears, but shortly after suspending antibiotic treatment the malodor usually reappears. In such cases tonsillectomy was usually elected. More recently, laser cryptolysis has become the treatment of choice for chronic caseous or non-caseous tonsillitis with presenting malodor (Freche, 1984). This is an office procedure without admission to the hospital. The procedure consists of superficial vaporization of the tonsils by CO_2 laser and complete tonsillectomy is avoided. If there is a large quantity of tissue to vaporize,

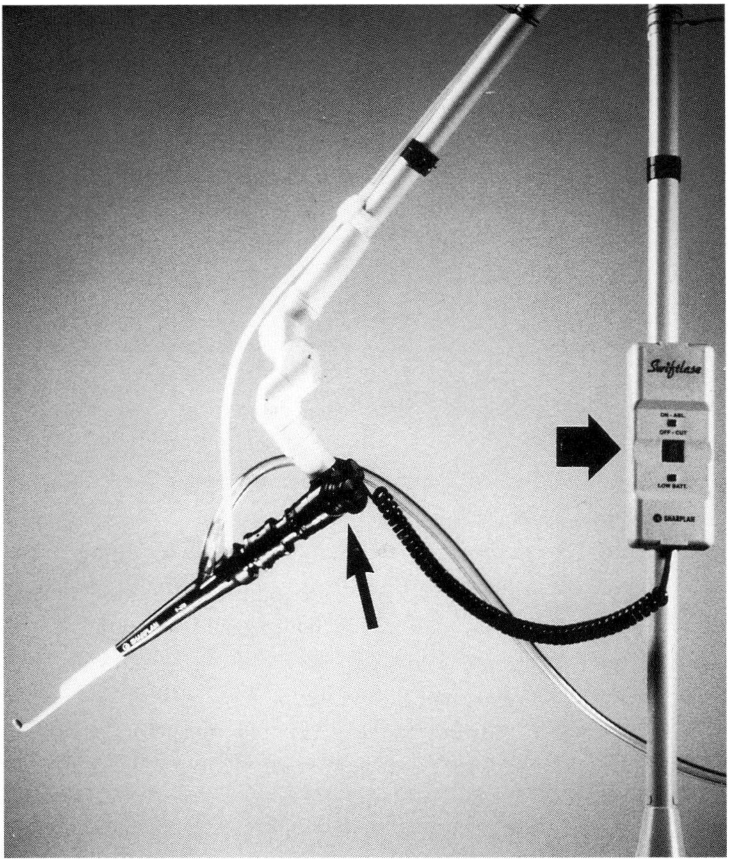

Figure 8. Sharplan SwiftLase 7566 Flashscan® and handpiece, assembled onto articulated arm. The optical head (thin arrow) is connected by the power control cord to the control box (thick arrow).

the procedure may be performed in two successive sessions separated by an interval of 3 weeks. The patient is placed in a seated position (Figure 6). Few patients require premedication. The local anesthesia is performed step-by-step and begins by spraying the oropharynx and oral cavity with 1.5% Lidocaine. Then a local infiltration of the tonsillar pillars from lateral medially and superiorly is made by a mixture of 1% Lidocaine and 0.001% Adrenalin, or of 2% Lidocaine and 0.01% Nordefrin. These mixtures are

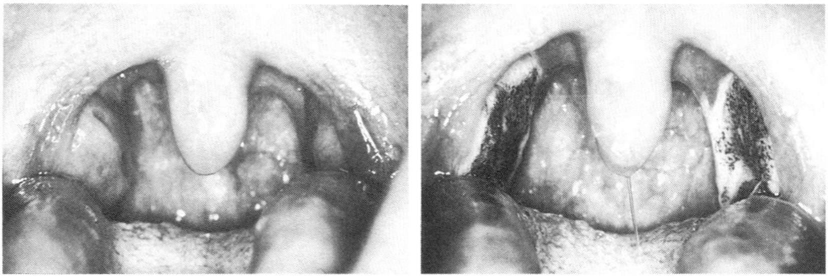

Figure 9. Peroral view of "innocent appearing" tonsils prior to (left) and following (right) laser cryptolysis.

available in cylinder ampullae for use with dental syringes and are easy-to-use. After infiltration of the tonsillar pillars the tonsils are also infiltrated. Accurate infiltration of the pillars is very important and may be almost sufficient *per se*. I use Sharplan's office ENT CO_2 laser system. This includes a portable 30 W CO_2 laser with smoke evacuator, and Sharplan 15251 Oral-Pharyngeal Delivery system. This system includes the laser's luminous guide-spot (Figure 6) mounted with an angled mirror tip (Figure 7) which deflects the laser energy to access and treats the tonsils under comfortable control, and SwiftLase® (Figure 8). The SwiftLase® scans the focused laser beam and delivers a 3 mm spot to the treatment site, rotating the energy so quickly over the surface area that the tissue is vaporized before any thermal damage or carbonization can occur. The system allows a progressive vaporization of the tonsils without extending the procedure beyond the tonsils and with minimal thermal damage to underlying tissue, which can aggravate the post operative pain. The power is used continuously between 10 and 20 W. The treatment is painless and lasts about 20 minutes, and there is no bleeding. After treatment, the patient may have a "sore throat" which is well controlled by analgesia and anti-inflammatory drugs. Figure 9 illustrates the effect of laser cryptolysis.

In summary, the tonsils of every patient complaining of halitosis have to be carefully examined, including squeezing to check for odorous discharge. When tonsil odor is deemed to contribute to the overall oral halitus, laser cryptolysis appears to provide an effective, safe and cost-effective treatment.

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12

Halitosis in Patients with Craniofacial Anomalies

Yehuda Finkelstein

Halitosis can originate from the nose, oral cavity, or upper digestive tract. In a vast majority of cases, bad breath has an oral etiology, although sinus and other nasal problems may be responsible for non-oral etiologies of halitosis (Finkelstein, chapter 11, this volume; Rosenberg and Leib, this volume). The otolaryngologic diagnostic and therapeutic approach to the upper respiratory tract is becoming increasingly based on endoscopic assessment. When necessary, endoscopic assessment is followed by computerized tomography (CT) to obtain a complete three-dimensional imaging of the upper respiratory tract including the paranasal sinuses. Relying on this modern view, we can discuss halitosis in patients with craniofacial anomalies.

Nasal sinusitis is frequent in patients with cleft palate. Deviated nasal septum, deformed medial turbinate, underdeveloped maxillary sinuses, maxillary growth deficits, choanal stenosis or atresia, atresia of the nostrils, malformed external nasal valve, incompetent velopharyngeal valve, presence of pharyngeal flap and impairment of mucociliary transport have all been suggested as reasons for the high incidence of sinusitis in patients with cleft palate (Drettner, 1960; Foster, 1962; Aduss and Pruzansky, 1967; Finkelstein *et al.*, 1990).

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Malformed intranasal structures including the nasal septum and middle turbinates are, in my opinion, the main cause of sinusitis in cleft palate patients. In these patients, the nasal cavity may be narrow because of maxillary hypoplasia. The nasal cavity may be completely occluded by severe deviation of the nasal septum. Prominent vomerine spurs penetrating the middle meatus and occluding the maxillary ostium are more frequent than in the non cleft population. Abnormal middle turbinates, bullous, paradoxically bent and obstructing the maxillary ostium are commonly observed. In addition to the well-known abnormalities, bizarre malformations of the middle turbinates may be observed. On the side of the cleft palate, the middle turbinates may by displaced laterally, compressed between the severely deviated septum and the lateral nasal wall in a narrow nasal cavity. In these patients, the endoscopic examination can be performed only on the contralateral side. Observing the nasopharynx through the patent nasal cavity, massive purulent postnasal drip from the contralateral choana flowing from the anteriorly occluded nasal cavity is found. Although chronic sinusitis and nasal obstruction are common in these patients, only some of them present with halitosis. On examination, in addition to the postnasal drip, the dorsum of the tongue is coated.

Many of these patients need correction of both the external nose and septum deformities as a first step to achieve acceptable nasal passages and nasal breathing. In the second step, they need endonasal endoscopic surgery to resolve their chronic sinusitis. In addition to the commonly encountered sinusitis, these patients may present special dilemmas. For example, the nasal cavity may be contaminated by saliva and food which pass from oral to nasal cavity through oronasal fistula, in which instance the fistula has to be closed. In the present chapter, three illustrative cases of cleft palate patients presenting with halitosis of unusual origins are discussed.

Patient No. 1

A 22-year-old woman with cleft palate was referred to us because of persistent severe halitosis which began after she underwent pharyngeal flap surgery 14 months previously (Finkelstein *et al.*, 1993). The fetid odor was so offensive that her marital and social life were totally disrupted. Previous consultations with otolaryngologists, plastic surgeons and general practitioners were not helpful.

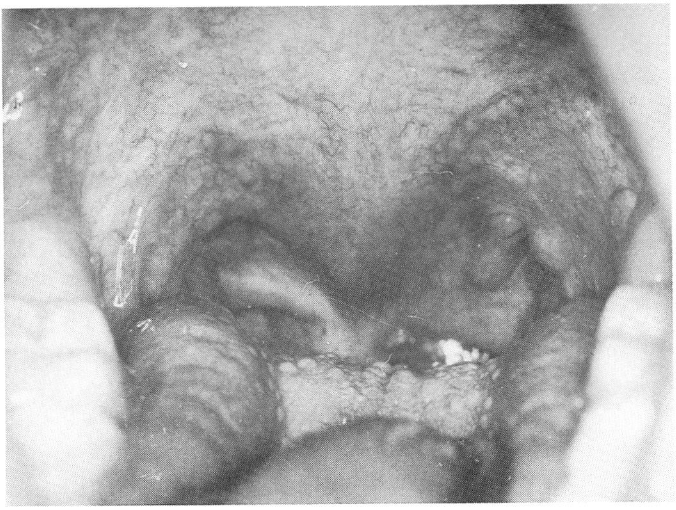

Figure 1. Peroral view of the patient with pharyngeal flap. Peroral direct or indirect examination of the nasopharynx is impossible (Courtesy of Plastic and Reconstructive Surgery).

On examination, a severe offensive fetid odor was perceived as emanating mainly from the nose, but there was a component from the oral cavity as well. Her speech was normal with mild hyponasality. On peroral examination a low flap obliterating the velopharyngeal isthmus from below was seen, preventing any direct or indirect mirror examination of the nasopharynx (Figure 1). Nasendoscopic examination revealed the presence of a grayish foreign body within the nasopharynx lying on the flap (Figure 2). The foreign body was immediately removed by endoscopic telebiopter, mounted with a rigid forward oblique endoscope (Figure 3), and revealed itself to be a gauze pad forgotten during the pharyngeal flap surgery (Figure 4). Removal of the gauze pad was followed by immediate disappearance of all symptoms.

Pharyngeal flap surgery is the most common secondary palate surgery. In these patients the nasopharynx is occluded from below by the pharyngeal flap. This patient posed a special challenge because usual otolaryngologic examination of the nasopharynx, either indirectly employing a mirror, or directly using a retractor or finger, was impossible. Before the endoscopic

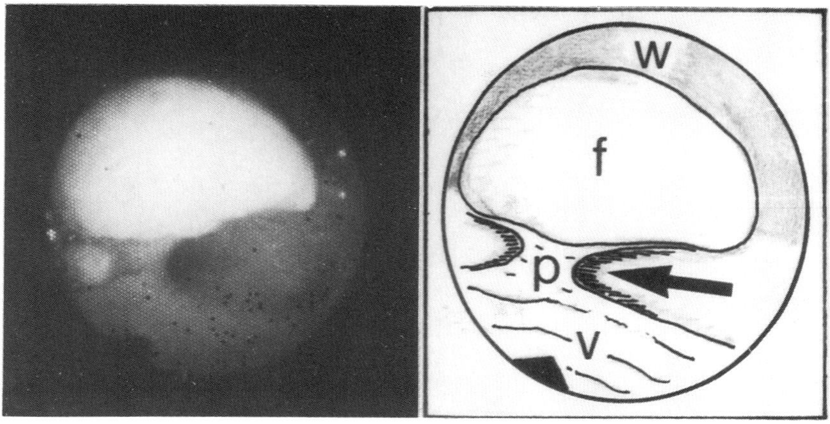

Figure 2. Nasendoscopic view of the foreign body in the nasopharynx lying on the pharyngeal flap. f: foreign body; p: pharyngeal flap; v: velum; w: posterior nasopharyngeal wall. The left port is indicated by an arrow (Courtesy of Plastic and Reconstructive Surgery) .

era, this patient probably would have undergone a difficult examination under general anesthesia. The removal of the gauze pad would also have been difficult and might have necessitated cutting the flap. Modern endoscopic equipment allowed both quick diagnosis of this very rare complication and immediate and simple treatment under topical anesthesia.

Patient No. 2

A 45-year-old male attorney had a 2-year history of intense malodor due to unilateral, purulent fetid rhinorrhea. The fetid odor was so offensive that his marital, professional and social life were totally disrupted. His medical history revealed that he had been born with left cleft palate and lip. Cleft lip repair was performed several months after birth. He underwent palatal repair at the age of three. From that time the patient was asymptomatic and his speech was normal. A short period before the onset of the halitosis he underwent dental treatment. The treatment included extraction of an unerupted maxillary left canine and fixed metal and porcelain (ceramic) prosthodontic partial denture.

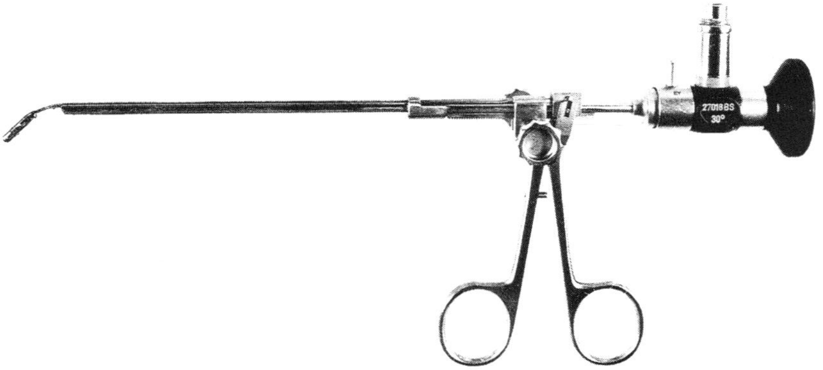

Figure 3. The endoscopic telebiopter (Storz Hopkins flexible optical biopsy forceps 723400), mounted on a Storz Hopkins (723400) rigid forward oblique 30°, diameter of 2.7 mm.

Examinations performed by dentists, maxillofacial surgeons and otolaryngologists were all unsuccessful in determining the cause of the patient's halitosis. The numerous plain sinus radiographs consistently showed a mild thickening of left maxillary sinus with a round-shaped polypoid mass on the floor of the sinus. The patient underwent various treatments which all failed.

On examination, although the patient complained about severe oral malodor, the offensive fetid odor was perceived as emanating mainly from the left nasal cavity. His speech was normal. The left nasal cavity was remarkably narrowed because of a deviated nasal septum and prominent maxillary crest, combined with the deformed left ala nasi. A small quantity of purulent discharge was observed in the left nasal cavity. The narrow slit form of the left nasal cavity prevented its endoscopic examination. A flexible nasendoscopic examination under topical anesthesia revealed that the right maxillary ostium and ethmoidal structures were normal. Advancing the nasendoscope, a small quantity of postnasal purulent discharge was observed flowing from the left choana. Following treatment with metronidazole

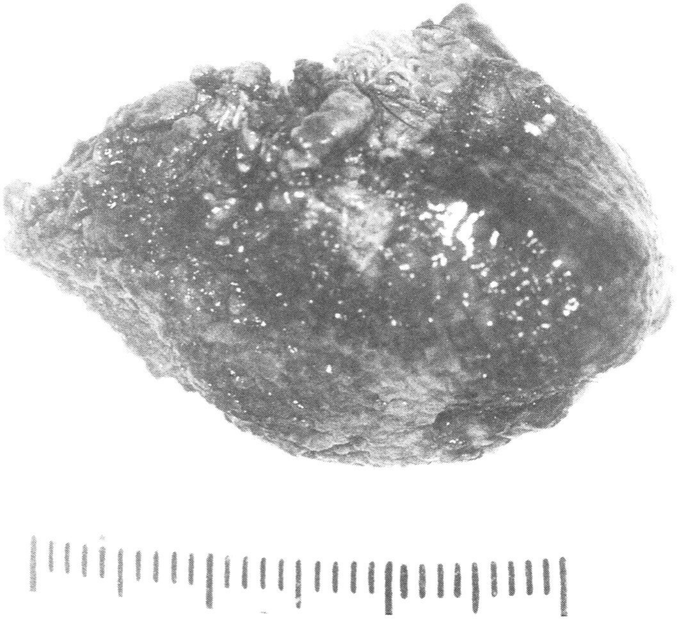

Figure 4. The removed gauze pad (4 cm length), (Courtesy of Plastic and Reconstructive Surgery).

and cephalexin monohydrate the halitosis disappeared but reappeared after cessation of treatment. A trial with Amoxycillin (as trihydrate) combined with clavulanic acid (augmentin) had a similar result. Computerized tomography revealed a defect in the anterior alveolar bone on the left continuing into the left hypoplastic nasal cavity (Figures 5 and 6). The nasal septum was deviated to the left with soft tissue filling the left nasal cavity. Soft tissue with a diameter of 1.5 cm was noted in the floor of left maxillary sinus possibly representing a cyst or polyp (Figure 6).

Puncture and lavage of the left maxillary sinus was performed and a small quantity of putrid purulent discharge was aspirated. A transient improvement was observed. The patient was hospitalized and underwent daily lavages of the maxillary sinus. He was discharged after 7 days and continued with oral antibiotics. Despite the rigorous treatment, halitosis recurred following termination of the antibiotic regimen.

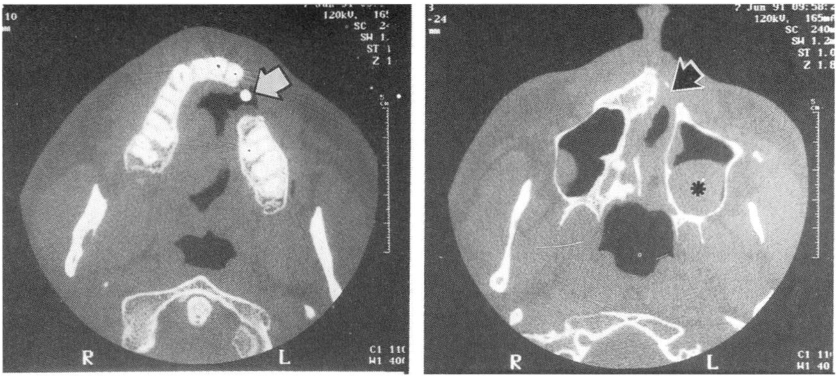

Figure 5. Axial section computerized tomography. The defect in the anterior alveolar bone with a part of the ceramic denture (white arrow on the right) is on the left, continuing into the left nasal cavity (black arrow on the left). Polypoid soft tissue in the left maxillary sinus cavity is indicated by asterisk on the right.

Panoramic dental computerized tomography did not reveal an oro-antral fistula (Figure 6). The patient was referred for re-examination by a maxillofacial surgeon who excluded an odontogenic etiology for the halitosis. The patient underwent submucous resection of the deviated nasal septum and the prominent maxillary crest combined with left inferior turbinectomy. The patient reported an improvement in the patency of the left nostril, but the halitosis remained unchanged.

The next step was a Caldwell-Luc operation. Intra-operatively, mild edema of maxillary sinus mucosa and a wide-based polyp the on antral floor were excised. A naso-antral window was made. On histologic examination a papilloma was diagnosed. However, inverted papilloma was excluded. Again, only a transient improvement was observed. One month post-operatively, endoscopic examination revealed a bluish material deep within the floor of the naso-antral window. This was immediately removed by a Storz Hopkins telebiopter (Figure 3). The foreign body, a bluish material, was removed. The object appeared to be a semicolloidal polymer, Xantopren which was apparently injected by the dentist as impression material for preparing the dental implant two years previously. Removal was followed by the immediate and permanent disappearance of all symptoms.

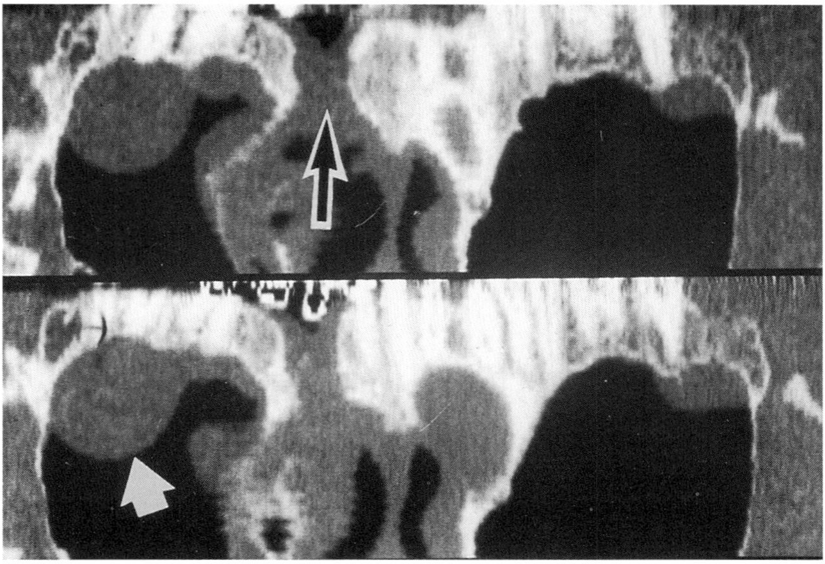

Figure 6. Panoramic dental computerized tomography showing the defect in the anterior alveolar bone continuing into the left nasal cavity (black arrow) and polypoid soft tissue on the floor of the left maxillary sinus (white arrow).

Xantopren is widely used by dentists as an impression material for preparing the dental implant. In this case it was injected after extraction of an unerupted maxillary left canine into the soft tissue filling the cleft where it remained as a foreign body. This material does not include any radiopaque component and therefore could not be found even in the meticulous CT scanning. During the operation it could not be seen from the outside and was only discovered following endoscopic examination of the oro-antral window and maxillary sinus from within. The endoscopic telebiopter facilitated its immediate removal.

Patient No. 3

An 18-year-old male patient was examined because of a 4-year history of offensive persistent fetid oral malodor accompanied by right purulent rhinorrhea. Medical history revealed that he had been born with right cleft palate and lip. Cleft lip repair was performed with excision of the pre-maxilla several months after birth. He underwent palatal repair at the age of two. Previous examinations by general practitioners, otolaryngologists and plastic surgeons were not helpful. He was also examined by an allergologist; allergy skin tests were found to be negative. The patient underwent serial antibiotic treatments which eliminated the malodor for short periods only. On the anterior rhinoscopic examination, the right inferior turbinate was edematous and hyperemic. On endoscopic examination, under putrid purulent discharge, an ectopic tooth was found in the floor of the right nasal cavity (Figure 7). The tooth was affected by caries and thus the suppuration and malodor were due to its exposure in the nasal cavity. On a lateral cephalogram (Figure 8, left) and panoramic radiograph (Figure 8, right), it was found that the patient had partial agenesis of his dentition in the upper jaw. His premaxilla, including the 4 permanent incisors, was excised in previous surgery during early childhood. The deciduous canine in the upper right is still present. The ectopic toothwas the canine tooth, impacted in an inverted position in the upper right maxilla, exposed to the floor of nose. The crown of the tooth showed irregularity indicating resorption of the enamel, presumably due to caries (Figure 8).

The tooth was removed by buccal approach with the envelope flap and was followed by immediate disappearance of the halitosis. In this case as in many other instances, if endoscopic examination had been performed, years of suffering and prolonged unnecessary antibiotic treatments might have been prevented.

Comment

Putrid type halitosis is a part of the spectrum of clinical diseases which involve the upper respiratory tract. The great dilemma with the majority of the patients, particularly with craniofacial anomalies, is whether or not the site of the infection is in the nose, sinuses, pharynx, tongue, oral cavity or elsewhere. Putrid odor from the nose may be due to aerobic chronic sinusitis. It is important to note that even in those patients with rhinosinusal origin of their malodor, an oral component of offending breath may be detected and thus be misleading. It must be emphasized that most of the purulent discharge produced in the sinuses flows posteriorly above the soft palate onto the base of tongue and hypopharynx. It then enters the esophagus and thus is swallowed. We still do not know how this influences the microbiota on the base of the

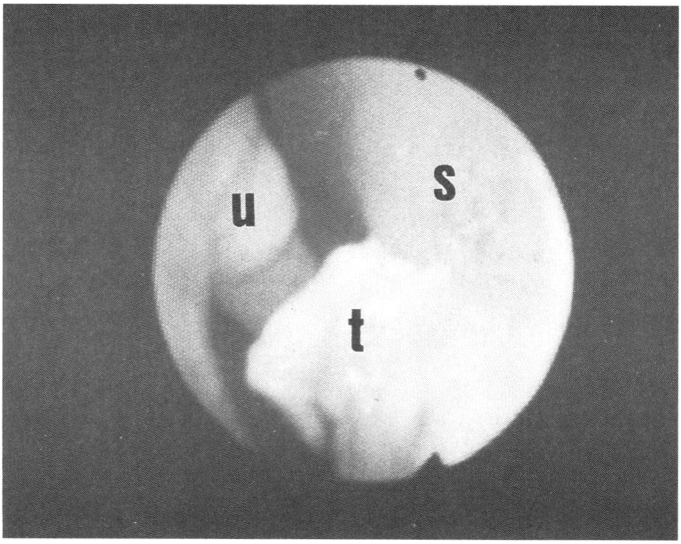

Figure 7. Endoscopic view of an ectopic tooth in the floor of the nasal cavity (arrow). The crown of the tooth is irregular because it was affected by caries.

tongue and affects the malodor (Rosenberg and Leib, this volume).

Normal or mildly abnormal rhinoscopic anterior examination and normal plain sinus radiographs are usually insufficient in halitosis investigation. Endoscopic examination is mandatory in patients with putrid breath. If signs of infection persist despite the medical treatment, computerized tomography of the paranasal sinuses must be done. In patients with craniofacial anomalies, panoramic mode computerized tomography is of particular importance for detecting oronasal or oroantral fistulas as well as unerupted teeth.

The present chapter discusses three cases of patients with cleft palate who suffered from halitosis due to rhinosinusal infections of specific causes. These cases illustrate the need to understand the special clinical aspects of those patients with craniofacial anomalies and the need to evaluate them thoroughly. In general, due to various malformations of the intranasal structures, chronic paranasal sinusitis, eventually leading to halitosis, is observed more

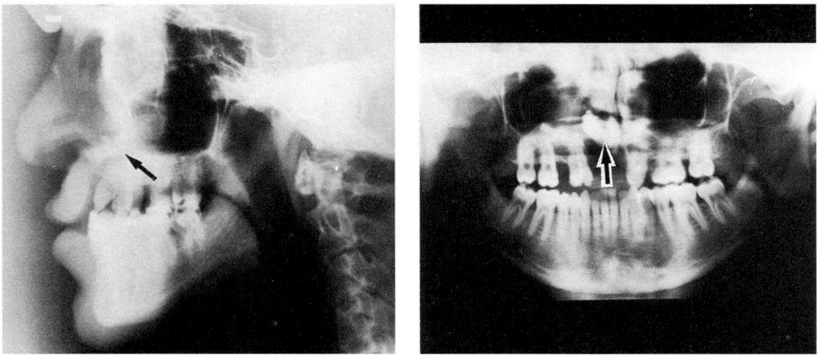

Figure 8. Lateral cephalogram, on the left and CT panoramic view on the right. An ectopic tooth (arrow) which is the deciduous canine impacted in inverted position in the upper right maxilla, exposed to the floor of nose (Courtesy of Shlomo Kalderon, MD, Head, Department of Maxillofacial Surgery, Beilinson Hospital, Petah Tikva, Israel).

frequently than in the normal population. Nasal obstruction or high nasal airway resistance leading to habitual mouth breathing is more common in patients with craniofacial anomalies because of deficient of maxillary development, and because of malformed external nose and intranasal structures. Poor dental status is also more frequent in cleft palate patients. Oronasal fistulas which may lead to continuous contamination of the nasal cavity are characteristic anomalies which may be found in these patients. The possibility of finding an ectopic tooth is higher than in a normal population. Dentists should be aware of the special aspects in cleft palate patients. Foreign bodies should be considered whenever purulent rhinorrhea or a foul odor emanates from the nose. Intranasal foreign bodies are frequently seen in pediatric patients (Fagan and Mathewson, 1990). They usually present with purulent rhinorrhea and a foul odor emanating from the affected nares. However, even in the adult population, particularly in patients with craniofacial anomalies, the possibility that halitosis may be related to a foreign body should be considered whenever malodor of nasal origin is found.

In summary, patients with craniofacial anomalies (particularly cleft palate patients) who complain of malodor should be subjected to rigorous evaluation, including a thorough endoscopic examination. Modern endoscopic equipment allows both quick diagnosis and immediate removal of foreign bodies under topical anesthesia.

Acknowledgment

I am grateful to the Editor, Plastic and Reconstructive Surgery, for permission to report findings previously published in Finkelstein *et al.* (1993) and to reproduce Figures 1 and 2.

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13

Psychological Factors in Self Assessment of Oral Malodor

Ilana Eli,[1]* Roni Baht[1] and Mel Rosenberg[1,2]

In many cases, complaints of oral malodor cannot be substantiated by physical examinations. As in other subjective perceptions, the personal perception of odor is highly affected by emotional and cognitive variables. In the present study a psychopathologic evaluation was carried out on 35 patients who arrived with a complaint of oral malodor to a multidisciplinary team formed to evaluate the problem. Patients were requested to evaluate their own malodor in several ways and to complete the SCL-90 questionnaire as a general measure of psychopathology. The clinical profile of patients was computed according to the following SCL-90 dimensions: somatization, obsession-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism. Results were compared with psychopathologic profiles of different groups of dental patients. The data suggest that patients with a subjective complaint of oral malodor present a unique group as compared to other groups of dental patients and that the subjective perception of oral malodor is related to psychopathologic trends which can be estimated by the SCL-90 questionnaire.

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Introduction

Personal concern over body odors is a major aspect of human nature, reflected in the popularity of commercial deodorants and mouthrinses. Among body odors, oral malodor (halitosis) is a major concern. The complaint of oral malodor is one of the main reasons that adults seek dental counsel (Tonzetich, 1977). Many people feel that they suffer from the problem sporadically (*e.g.* at awakening or after consumption of special food); others claim to suffer from halitosis at all times to the degree that it interferes with their social and professional life.

In many cases halitosis does have a physiological etiology originating from conditions, such as poor general oral hygiene, periodontal disease, dry mouth, food impaction, improper or faulty restorations, unclean dentures, microbial activity on the dorsum of the tongue, throat infections and oral carcinomas (Tonzetich, 1977; Rosenberg, 1990). Sometimes, however, the subjective complaint of oral malodor originates due to presumptions based on others' attitudes (Iwakura *et al.*, 1994).

As with other subjective perceptions (sight, hearing, pain, etc.), smell is affected by emotional, as well as cognitive variables. In recent years a significant body of literature has emphasized the role of olfactory system in memory and cognition. On the one hand, human beings possess an exquisite capacity to detect and distinguish odorants. On the other hand, identifying particular odorants may sometimes pose problems (Richardson and Zucco, 1989). The perception of smell is strongly associated with various affective states and thus can influence, and is influenced by mood (Ehrlichman and Halpern, 1988).

Central to successful diagnosis and treatment of halitosis is the recognition that the patient's subjective complaint is not necessarily associated with objective parameters (Rosenberg and Leib, this volume). A chronic ("unjustified" or "justified") complaint of halitosis may originate in poor body image. The body image (a central representation of the body parts, smell, voice and the body as a whole) has a significant effect on the individual's self-image and social role. Its development starts at the early stages of childhood when there is no distinction between the self, the body, the body smell and excrements. Normal personality development leads to differentiation between the body and self-image, but when psychopathologic factors interfere with the process diffusion among the parameters may occur.

The perception of body odor plays a significant part in the development of the body image. Some people who really suffer from repellant body odor may remain unaware of it unless the problem is pointed out by significant others. Others may be overanxious about having a potential smell of sweat or oral malodor to the point of developing of a delusion of bad smell. The development of delusion usually has deep, unconscious roots and can significantly interfere with the individual's personal, professional and social life. In a delusion disorder of the somatic type, it is often found that the patient is convinced of having malodor from the skin, mouth, rectum or vagina. Such patients typically consult specialists in the specific fields, rather than a psychologist, and search for physiological solutions for their problem.

Pryse-Phillips (1971) was the first to describe an "olfactory reference syndrome" in which the patient claims to actually perceive the bad odor that others cannot detect. The syndrome was characterized by the following features: young age at onset, male predominance, no prior history of psychiatric treatment, a search for medical solutions, preservation of integrated personal and social life, albeit dominated by the syndrome, a strong contrite reaction and the intrinsic nature of abnormal perception. Cases of delusional halitosis were presented in the literature by Davidson and Mukherjee (1982), and by Iwo and Akpata (1989).

The lives of patients who believe (rightfully or not) that they suffer from oral malodor are often strongly affected as a result (Rosenberg, 1990; Davidson and Mukherjee, 1982). A better understanding of the physical and/or psychological factors related to the development of such beliefs may lead to improved treatment modes for this complex problem.

Materials and Methods

Thirty five subjects (mean age 43 ± 10 years; 66% female) who responded to a newspaper article on halitosis were clinically and psychologically evaluated. On the day of the examination the participants were instructed to refrain from oral activities, including drinking, eating, chewing gum and mouth rinsing two hours prior to appointment. Subjects were also asked to refrain from using scented personal products.

Subjects were evaluated according to several clinical and biochemical parameters including plaque and gingival indices, mean probing depth, evaluation of log-transformed peak and steady volatile sulfide levels in the oral headspace, measurements of trypsin-like activity of samples from four oral sites and levels of odorous diamine in saliva. Data concerning these evaluations are reported elsewhere (Goldberg *et al.*, 1994; Kozlovsky *et al.*, 1994; Rosenberg *et al.*, 1995).

All subjects were asked to evaluate their own oral malodor on a 10 cm visual analog scale (VAS) (0 - no malodor; 10 - malodor as bad as one can imagine) according to the following parameters:

1. A general initial guess about the level of one's own malodor prior to any clinical examinations (preconception – PreC).
2. Self-evaluation of whole mouth malodor (WMM), by exhaling into one's hands which cupped over mouth and nose.
3. Self-evaluation of the smell created by licking one's own wrist (tongue malodor -TM).
4. Self-evaluation of the smell of one's own saliva expectorated into a petri dish 5-10 min earlier (Saliva Malodor – SM).
5. A general evaluation of one's own malodor following the clinical examinations (post-conception – PostC).

Concomitantly, all subjects were requested to complete the SCL-90 questionnaire developed by Derogatis *et al.* (1973) as a general measure of psychopathology in clinical and research situations. The questionnaire has shown high rates of validity and reliability in numerous clinical studies (Guy, 1976) and has been successfully used in clinical dental settings, such as in identifying psychometric profiles of craniomandibular pain patients, predicting the success of behavior therapy in the treatment of dental fear and avoidance and evaluation of patients suffering from Burning Mouth Syndrome (Butterworth and Deardorff, 1987; Deardorff and Butterworth, 1987; Kleinhauz *et al.*, 1992; Eli *et al.*, 1994a,b). The clinical profile of patients includes the following dimensions:

i. Somatization – reflects distress arising from perceptions of bodily dysfunction.

ii. Obsession-compulsion – reflects thoughts, impulses and actions that are experienced as unremitting and irresistible by the individual but are of an ego-alien or unwanted nature.

iii. Interpersonal sensitivity – focuses on feelings of personal inadequacy and inferiority, particularly when compared with other individuals.

iv. Depression – reflects a broad range of the concomitants of the clinical depressive syndrome (*e.g.*, symptoms of dysphoric affect and mood, signs of withdrawal of interest in life events, lack of motivation, loss of vital energy).

v. Anxiety – reflects symptoms and experiences usually associated clinically with high manifest anxiety (*e.g.*, restlessness, nervousness, tension).

vi. Hostility – reflects three categories of hostile behavior: thoughts, feelings and actions.

vii. Phobic anxiety – reflects symptoms that have been observed with high incidence in conditions termed phobic anxiety state (*e.g.*, fears of phobic nature oriented towards travel away from home, open spaces, crowds, public spaces or social phobic behavior).

viii. Paranoid ideation – scales developed around the primary characteristics of paranoid thought (*e.g.*, projective thinking, hostility, suspiciousness, centrality, delusions, loss of autonomy).

ix. Psychoticism – includes measures sampling psychotic behaviors from acute symptomatology to less definite indicators of psychotic processes.

Global scores of the questionnaire are presented under General Symptomatic Index (GSI), Positive Symptom Total (PST) and Positive Symptom Distress Index (PSDI).

Findings

The clinical psychopathologic profile (as evaluated by the SCL-90 questionnaire) of subjects with a complaint of oral malodor is presented in Figure 1. It shows some unique features, which may be characteristic of this population.

When compared to a general population of dental patients (age- and gender-matched), the patients with a complaint of oral malodor show marked elevations in the subscales of obsession-compulsion, interpersonal sensitivity, anxiety and psychoticism (Figure 1). Their scores on the scales of obsession-compulsion, interpersonal sensitivity, depression and psychoticism resemble the scores of dental phobics who have been treated successfully for their dental fear (Figure 2). Their psychopathologic profile runs almost parallel (but lower) to that of dental phobics who failed treatment through the mode of behavior modification (Figure 3). The scores of the halitosis patients on the scales of obsession-compulsion and paranoid ideation resemble the scores of patients who suffer from Burning Mouth Syndrome (Glossodynia)

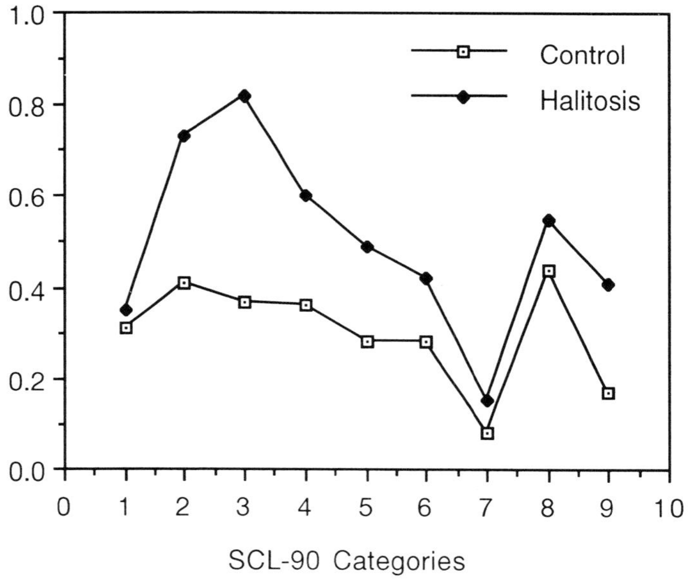

Figure 1. SCL-90 mean score profiles of patients as follows: halitosis – patients with a complaint of oral malodor as described in Materials and Methods; control – a general population of dental patients (age- and gender-matched) who have been under routine dental treatment in a dental affiliated clinic.

SCL-90 categories: 1 – Somatization; 2 – Obsession-compulsion; 3 – Interpersonal sensitivity; 4 – Depression; 5 – Anxiety; 6 – Hostility; 7 – Phobic anxiety; 8– Paranoid ideation; 9 – Psychoticism.

(Figure 4). When correlations were computed comparing the patients' psychopathologic profiles and variables of malodor self-assessment, significant associations were found between whole mouth malodor (WMM) and 9 out of 12 of the SCL-90 scales; between tongue malodor (TM) and 7 of the SCL-90 scales; and between post-conception (PostC) and 6 of the SCL-90 scales (Table 1). In contrast, saliva malodor (SM) and preconception (PreC) were associated with only one of the psychopathologic scores.

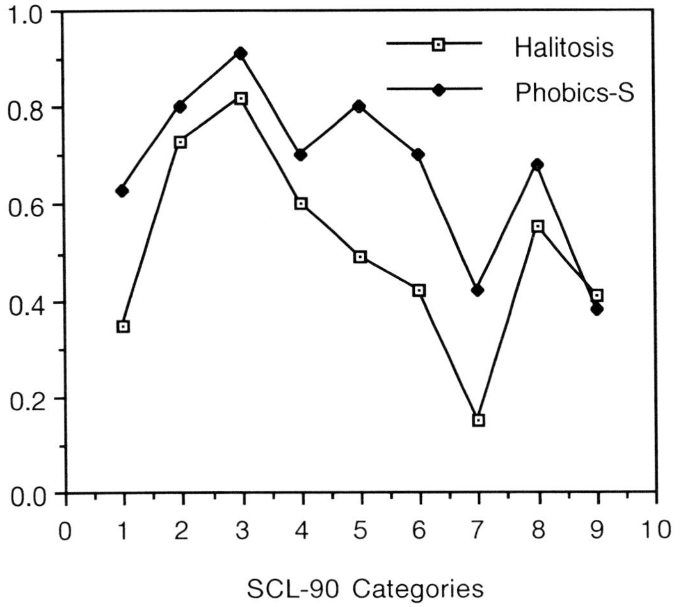

Figure 2. SCL-90 mean score profiles of patients as follows: halitosis – patients with a complaint of oral malodor as described in Materials and Methods; phobics-S – dental phobics who have been treated successfully for their dental fear (Kleinhauz *et al.,* 1992).

SCL-90 categories: 1 – Somatization; 2 – Obsession-compulsion; 3 – Interpersonal sensitivity; 4 – Depression; 5 – Anxiety; 6 – Hostility; 7 – Phobic anxiety; 8– Paranoid ideation; 9 – Psychoticism.

Discussion

The results of the present study indicate that the subjective perception of oral malodor is affected by, and may be the result of psychopathologic trends as recorded by the SCL-90 questionnaire. Patients who are highly aware of their oral odor may present a distinct psychopathologic profile, regardless of the reason which brings them to this conception.

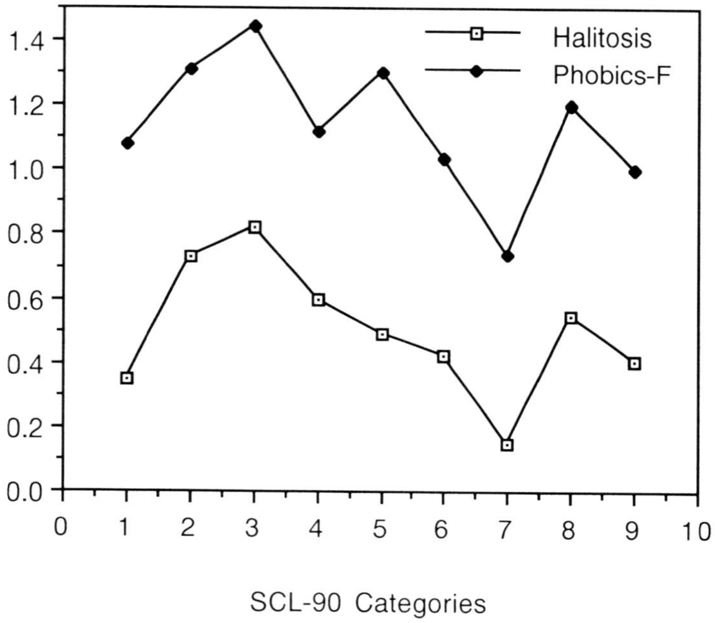

Figure 3. SCL-90 mean score profiles of patients as follows: halitosis – patients with a complaint of oral malodor as described in Materials and Methods; phobics-F – dental phobics who have failed treatment for their dental fear (Kleinhauz *et al.*, 1992).

SCL-90 categories: 1 – Somatization; 2 – Obsession-compulsion; 3 – Interpersonal sensitivity; 4 – Depression; 5 – Anxiety; 6 – Hostility; 7 – Phobic anxiety; 8– Paranoid ideation; 9 – Psychoticism.

Patients who arrive with a complaint of halitosis show significant mean elevations in their scores for obsession-compulsion, interpersonal sensitivity, anxiety and psychoticism when compared with a general population of dental patients matched for age and gender.

Although our initial hypothesis was that the complaint of halitosis may originate in somatization, the psychopathologic profile of patients does not support this premise. The psychopathologic scores of the halitosis patients indicate that they have a tendency to focus on feelings of personal inadequacy and inferiority and on thoughts, impulses and actions that are unremitting

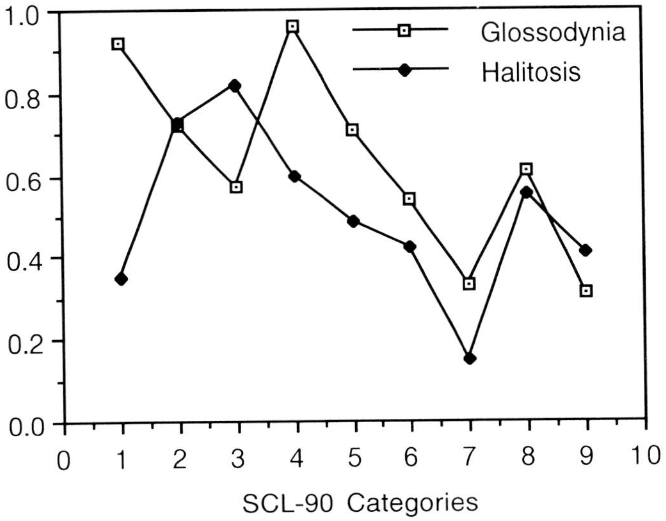

Figure 4. SCL-90 profiles of patients as follows: halitosis - patients with a complaint of oral malodor as described in Materials and Methods; Glossodynia - patients who suffer from Burning Mouth Syndrome (Eli *et al.*, 1994a,b).

SCL-90 categories: 1 – Somatization; 2 – Obsession-compulsion; 3 – Interpersonal sensitivity; 4 – Depression; 5 – Anxiety; 6 – Hostility; 7 – Phobic anxiety; 8– Paranoid ideation; 9 – Psychoticism.

and irresistible by the individual. In this respect they resemble another large group of dental patients who suffer from a different behavioral dysfunction related to oral care – the "dental phobics". Although common etiologic factors of dental fear/anxiety/phobia include environmental influences, such as direct learning through early aversive experiences in the dental office, some reports indicate specific personality traits which, when present, increase the patients's proneness to apprehension in the dental setting (Eli, 1992). The similar psychopathologic features between the "dental phobics" and "halitophobics" suggests an increased preoccupation with the bodily functions in both populations.

Table 1
Pearson Correlations between Self-Assessments and Psychopathologic Parameters

	PreC	**WMM**	**TM**	**SM**	**PostC**
Subscales:					
Soma.	r=0.28* p=0.05	0.33* 0.03	0.23 0.09	0.19 0.13	0.24 0.08
Obs.-Comp.	0.04 0.39	0.32* 0.03	0.26 0.06	0.08 0.31	0.27* 0.05
Int. Sens.	0.05 0.37	0.31* 0.03	0.27* 0.05	0.12 0.24	0.26 0.06
Depr.	0.07 0.33	0.34* 0.02	0.43** 0.00	0.04 0.41	0.38** 0.01
Anx.	0.04 0.40	0.25 0.07	0.37* 0.01	0.08 0.20	0.34 0.22
Host.	-0.00 0.49	0.15 0.19	0.24 0.08	0.27* 0.05	0.26 0.06
Phob.Anx.	0.24 0.08	0.29* 0.04	0.17 0.16	0.17 0.16	0.32* 0.03
Para.Id.	0.02 0.45	0.31* 0.03	0.41** 0.00	0.08 0.32	0.27* 0.05
Psych.	0.00 0.47	0.29* 0.04	0.42** 0.00	0.02 0.45	0.27* 0.05
Global Scales:					
GSI	0.05 0.39	0.34* 0.02	0.39** 0.01	0.10 0.26	0.34 0.27
PST	0.06 0.36	0.34* 0.02	0.34* 0.02	0.15 0.19	0.28* 0.04
PSDI	-0.05 0.38	0.18 0.14	0.20 0.12	0.03 0.47	0.21 0.10

*Significant correlations (P<0.05)
**Significant correlations (P<0.01)

Another finding is the tendency of the "halitosis complainers" to hostility and suspiciousness, which resemble dental patients who suffer from Glossodynia – a prolonged syndrome of chronic pain with no specific organic findings (Eli *et al.,* 1994a,b). As the personality profiles of the Glossodynia patients show mean elevations in depression, anger and anxiety, it was suggested that the psychological findings may be the consequence of the chronic pain condition rather than its cause (Grushka *et al.*, 1987). Although comparisons between populations of patients should be carried out with care (differences in population characteristics, sociocultural contexts, etc.), it might be that the increased psychological distress of the "halitosis complainer" is associated with their chronic condition in a similar manner to the Glossodynia patient. Both in Glossodynia and in self-perceived halitosis no clear-cut organic findings can usually be found to account for the syndrome. Many clinicians tend to classify such complaints as unimportant, "imagined" or practically "non-existent". The patient, however, faces continuous, chronic suffering with little hope for a possible cure. Anger, hostility, suspicion, and fear of loss of autonomy may be the inevitable consequences of such conditions.

The data suggest that the elevated psycho-pathologic profile of the halitosis patients is positively related to their self-evaluation of oral malodor. The positive correlations between most SCL-90 parameters (especially the general scores of GSI and PST) and self-evaluation of whole mouth malodor and tongue malodor suggest that psychopathologic factors confound the patients' "objectivity" when carrying out the self-assessments. The fact that only one correlation was found between SCL-90 scores and saliva malodor further supports this notion. Once the smell (saliva) is removed from the body proper to a petri dish it may enable a less "emotional" and more "objective" evaluation. This is supported by the finding that among the self-assessments, only the saliva self-scoring showed significant correlations with some of the laboratory parameters (data not shown).

The fact that psychopathologic scores were found to correlate with the patients post-conception (but not with preconception) suggests that focusing on the problem via induced self measurements increases the probability that patients higher on psychopathologic scores will evaluate themselves as having more malodor than before.

Fortunately, cases of somatic delusional halitosis appear to be rather rare.

However, an increased concern over oral and body odors is a common feature in Western society, supporting huge industries of mouth rinses, deodorants and cosmetics. The results presented here, together with data obtained in a malodor clinic (Rosenberg and Leib, this volume) are in agreement with the findings of Iwakura *et al.* (1994) who found that most patients with primary report of halitosis do not actually have halitosis but suffer from imaginary halitosis due to presumptions based upon others' attitudes.

The unique population of dental patients with a subjective, persistent complaint of oral malodor, presents a problem which has not, as yet, received enough attention from the dental community. Better understanding of the possible factors which contribute to the development of this problem is important in order to enable better coping modes with this complex issue.

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Appendix 1

Conference Report: First International Workshop on Oral Malodor

Mel Rosenberg

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The First International Workshop on Oral Malodor was held at the Daniel Hotel in Herzliya, Israel on April 26-28th, 1993. The meeting was hosted by the Sackler Faculty of Medicine, Tel Aviv University, and supported by a grant from the Warner-Lambert Company. The Workshop was attended by over 40 participants from Belgium, Canada, England, Israel, Japan and the U.S.A., representing a wide array of disciplines, including microbiology, dentistry, medicine, molecular biology, chemistry, psychophysics, psychology, and product development. The meeting included 18 oral presentations by researchers, clinicians, and industrial representatives, as well as a round table discussion.

At a Gala Dinner overlooking the Mediterranean, attendees were welcomed by the Rector of Tel Aviv University, the Vice-President for Research and Development, the Dean of the Faculty of Medicine and the Head of the School of Dental Medicine.

The scientific sessions of the Workshop were opened by a brief introduction by co-organizer Dr. Mel Rosenberg (Tel Aviv University) who acknowledged the pioneering and ongoing work of Dr. Joseph Tonzetich, University of British Columbia, in the field of oral malodor research. Dr. Jacob Gabbay

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(Ministry of the Environment, Israeli Government) served as the Chairperson of the first session, and introduced the first speaker, Dr. William S. Cain from the John B. Pierce Laboratory and Yale University. Dr. Cain's presentation dealt with problems associated with human assessment of odor mixtures. At barely detectable threshold concentrations, odors generally show simple additivity, but at higher levels, odors are invariably hypoadditive. This is due to brain processing, rather than altered receptor function. Our ability to discern the quality of an odor depends to some extent on cognitive processes, such as giving a label to a certain odor mixture. Such observations are relevant to the masking effect of oral hygiene products on oral malodor, as well as measurement of odor intensity and quality by judges. For example, the more complex the masking odor mixture, the better its masking ability. Mint, the odorant of choice in many anti-malodor products, may also mask oral malodor due to its pungent nature.

Hollandra P. Niles (Colgate-Palmolive) presented several interesting investigations dealing with measurement of malodor. She first summarized investigations demonstrating (i) the role of Gram-negative microorganisms in causing mouth odor; (ii) the relationship between mouth odor and gingivitis; and (iii) the relationship between sensory and instrumental evaluations, including sample storage. She described results of an experiment carried out on 20 male volunteers, showing a strong correlation between gingival crevicular fluid volume and hydrogen sulfide production. Ms. Niles also presented data comparing sensory and instrumental (gas chromatographic) techniques for evaluation of mouth odor. Mouth air samples were collected from twenty subjects in Telfon storage loops, which were subsequently transported to the laboratory in dry ice and analyzed by gas chromatography. Malodor was scored by two professional judges on a 0-8 scale. When subjects performed regular oral hygiene, only a poor correlation between sensory and instrumental analysis was observed ($r=0.22$). However, following subjects' use of a placebo dentrifice, the correlation improved ($r=0.77$).

Dr. Manny Shaw (President, Interscan Corporation) described the operating principles and data obtained using a portable sulfide monitor ("halimeter") which has recently been employed for intraoral measurement of volatile sulfides related to oral malodor. Dr. Shaw presented data on the linear response of the monitor to a range of hydrogen sulfide concentrations, showing that hydrogen sulfide and methyl mercaptan yield similar sensitivities, that the sulfide monitor reaches 90% response on a given signal within one minute, and that sequential measurements of whole mouth malodor from individuals show good repeatability. One enigma which was discussed was the negative deflection observed in some measurements. Several alternative sampling methods were also suggested by some of the participants.

Dr. Israel Kleinberg (State University of New York at Stony Brook) began his presentation by reviewing some of the pioneering saliva research work. When glucose is added to saliva samples, one obtains an acid pH, gram positive bacteria emerge, and little odor is produced. However, if one leaves out the glucose, an alkaline pH is obtained concomitant with a larger population of gram negative microorganisms and a putrid odor, presumably due to proteolysis and aminolysis. Dr. Kleinberg described metabolic function grids which characterize the predominant bacteria in supragingival plaque and summarized the effects of various substrates (*e.g.* amino acids) involved in malodor formation over time. From a study of the grid, it is evident that fusiforms, *Porphyromonas gingivalis* and *Prevotella intermedia*, are potent odor formers, with *Veillonella* and *Haemophilus* providing some malodor. Among the amino acids, not only sulfur-containing amino acids contribute to saliva malodor, but also ornithine and tryptophan. Finally, Dr. Kleinberg described modification of the halimeter for in vitro saliva putrefaction assays.

Dr. Walter J. Loesche (University of Michigan, Ann Arbor) discussed strategies to identify the main microbial contributors to oral malodor, emphasizing the potential role of tongue microbiota. He described initial attempts to link the BANA test, odor judge scores and volatile sulfide levels. Dr. Loesche proposed measuring oral malodor using the halimeter at 30 and 60 second time points, and described differences observed in certain subjects. He also commented on the inability to pinpoint specific microbial species that appear to be responsible for oral malodor.

Sarit Goldberg (Tel Aviv University) presented initial evidence suggesting that cadaverine, a volatile diamine produced during microbial putrefaction, may be an additional component of oral malodor. Highly significant correlations were found between the level of cadaverine in saliva samples, and oral malodor parameters in a population of 52 subjects. Incubation of saliva in vitro also led to an increase in cadaverine concentration which was concomitant with malodor elaboration.

Dr. Hideo Miyazaki (Kyushu Dental College) reported an epidemiological survey on oral malodor in 2601 Japanese adults aged 18-64, based on sulfide monitor readings. Findings included a trend towards higher levels of volatile sulfur compounds (VSC) in males, increases in VSC with increasing age, and an association between oral malodor and tongue coating or periodontal conditions. One-quarter of all subjects examined had VSC values of over 75 ppb, a value which might be considered as an acceptable/unacceptable cut-off level.

Dr. Mel Rosenberg described a population of 317 subjects who attended a malodor clinic during 1992. Odor judge scores of this population were distributed in a Gaussian fashion, with a significant proportion of subjects having only slight odor. When 93 women were asked to rate their own bad breath, 29% scored themselves at the highest possible level, whereas actual scores were much lower. Six classes of distinct, readily distinguishable odors recurred frequently: (i) odor typical of subgingival putrefaction; (ii) odor from the anterior of the tongue dorsum; (iii) odor from the posterior of the tongue dorsum; (iv) nasal odor; (v) denture odor; and (vi) smokers' odor. He concluded that future investigations should consider not only the intensity, but also the quality of oral malodor.

Dr. Christopher A.G. McCulloch (University of Toronto) addressed the question: "Is there a quantifiable relationship between periodontitis and oral malodor which may lead to a simple screening procedure of value in early diagnosis of periodontal diseases?" Data obtained using the sulfide monitor showed that replicate measurements made one week apart, were mostly within one standard deviation of the mean, providing evidence that the monitor provides fairly repeatable data. In a study of 127 subjects, correlations were found between sulfide monitor VSC measurement and odor judge scores, but VSC levels did not appear to be associated with plaque index, gingival index, nor presence of deep pockets. Dr. McCulloch also described the effect of chlorhexidine in reducing tongue malodor, concomitant with a mean reduction in tongue dorum pH of the order of 0.7 units. Dr. McCulloch concluded that the data obtained at the University of Toronto did not support use of oral malodor parameters as a screening test for the presence of periodontitis.

Dr. Rosenberg read the paper of Dr. Avital Kozlovsky (Tel Aviv University) on the association between oral malodor parameters and dental parameters in an Israeli subject population (N=52). In addition to VSC, whole mouth, tongue and saliva malodor were each assessed. In contrast to the Canadian study described above, highly significant correlations were found between all malodor parameters and plaque index, gingival index, and pocket depth. Discussion centered around potential reasons for the apparent discrepancy in these two studies.

Dr. Zeev Ram (Rehovot, Israel) presented data on the effect of initial periodontal preparation on halimeter measurements. Among 47 patients, a highly significant ($p<0.001$) mean reduction of 35% in volatile sulfides was found. These data support the premise that improvement in periodontal health is associated with an overall reduction in oral malodor.

Dr. Ken Yaegaki (Tsurumi University) described the initial stages of a project designed to develop a user-friendly bad breath detector which is superior to those currently employed, and equivalent to gas chromatography. The prototype has six conductor sensors each having a different sensitivity. This instrument discriminates between foul and pleasant odors. Dr. Yaegaki also provided evidence showing the importance of tongue coating in oral malodor, and the elevated level of volatile sulfur compounds produced by tongue coatings of patients with periodontal disease.

Dr. George Preti (Monell Chemical Senses Center, Philadelphia) focused on a group of patients complaining of oral malodor who were diagnosed as having trimethylaminuria. This metabolic disease, which results in overproduction of trimethylamine, an odorant with the smell of rotten fish, was once thought to be relatively rare, but is perhaps more prevalent than previously considered. Whereas trimethylamine has an extremely foul odor, patients rarely give off this smell. Dr. Preti suggested that the presence of trimethylamine in the saliva of such patients causes them to perceive that they suffer from oral malodor.

Dr. Yehuda Finkelstein (Meir Hospital, Israel) discussed halitosis in patients with craniofacial anomalies. Dr. Finkelstein suggested the sinuses as a potential site of halitosis, and stressed the importance of endoscopic examinations in seeking pathologic involvement. He presented the case of a woman whose halitosis derived from a foreign body (gauze pad) forgotten during pharyngeal flap surgery, and discovered 14 months later during endoscopic examination. In another patient, dental impression material was injected into the soft tissue filling a cleft palate, beneath the gingiva. In yet another instance, halitosis was found to derive from an ectopic tooth present in the patient's nasal floor.

The round table discussion, moderated by Dr. Loesche, focused on several questions related to current and future oral malodor research. Should a multicenter study on malodor be set up (as suggested by Dr. Daniel van Steenberghe) which would help clarify relationship between periodontal diseases and bad breath? Should meta analysis of existing data be done first in order to refine the research directions (Dr. McCulloch)? What are the principles guiding self-assessment of oral malodor (Dr. Cain)? Are subjects guided according to mouth taste (H.P. Niles, J. McDonough)? Is self-perception of oral malodor largely based on physiological cues (Dr. I. Kleinberg) or does it involve a combination of physiological and psychological parameters (Dr. I. Eli)? Are genetic factors (*e.g.*, hyperosmia) involved in variation among judges (Dr. D. Lancet)? Should hyperosmic smellers be recruited as malodor judges (Dr. Loesche)? Should more use be made of smell chromatography to identify specific odorants other than volatile sulfur components (Dr. Preti, Dr. Cain)?

The round table discussion also addressed the type of research that should be conducted in this area. As Sy Weinstein (Warner-Lambert Co.) pointed out, oral malodor in the business world is treated primarily as a cosmetic issue. Brenda Schwartz (Warner-Lambert Co.) commented on the subject populations used in malodor research. She described a national telephone survey indicating that about 60% of American women and 50% of men use cosmetic breath freshening products (candies, chewing gums, sprays, etc.). Many of these are people who like to indulge in products which they think are going to give them bad breath (coffee, pizza). Such individuals appear to account for the majority of those concerned about bad breath, yet may be underrepresented in clinical studies involving self-presenting subjects (Dr. S. Harper).

Dr. Doron Lancet (Weizmann Institute of Science) provided a molecular level view of human smell. He explained the ability of the olfactory system to discriminate millions of odorants, as being based on several hundred G-protein coupled olfactory receptors. Each receptor binds odorants with a different affinity (or not at all) yielding a pattern representative of the particular odorant. Work in Dr. Lancet's laboratory has shown that the odorant signal is apparently terminated by an enzyme which renders the odorant molecules nonodorous. The genes endoding the odorant receptors are clustered in an "olfactory sub-genome", and about 20 receptors have already been cloned. These studies provide the basis for seeking molecular differences which may correspond to the differences in which individuals perceive various odorants.

Dr. Ilana Eli (Tel Aviv University) described some of the psychological factors leading people to believe that they suffer from oral malodor. A psychological evaluation was caried out using the SCL-90 questionnaire developed by Derogatis in 1973. Subjects (N=35), presenting for oral malodor, were compared with 45 patients who presented for dental work. The oral malodor group had elevated scores in all categories of the psychological profile, particularly with respect to obsession and personal sensitivity. When the oral malodor subjects were asked to score their own malodor by cupping their hands over nose and mouth, or by licking their tongue and subsequently smelling their wrist, a positive correlation was found between self-given scores and the psychological questionnaire. The data underlie the importance of psychological factors, such as body image, on how individuals perceive their own oral malodor.

In his closing talk, Dr. Scott Harper (Warner-Lambert Co.) asked the "who, what, where, when how, why and so what?" questions regarding oral malodor. Dr. Harper defined four groups of individuals: (i) they have it and don't know; (ii) they don't have it and don't care; (iii) they have it and they know about it; and (iv) they don't have it but think they do. He stressed the possible association between mouth taste and perceived smell, and reviewed possible avenues of antimalodor product development.

When this First International Workshop on Oral Malodor was organized, an attempt was made to concentrate on measurement aspects, leaving the issue of treatment largely aside. This would make a good (albeit controversial) subject for a Second Workshop. A related matter, which was largely unresolved, was the degree to which malodor should be treated (by both academia and industry) as an intrinsic condition requiring medical attention, or rather as a cosmetic problem related to consumption of odorous foodstuffs, alcohol or cigarettes. As a case in point, what subject populations should be used to test the efficacy of breath freshening products? Finally, just as periodontal diseases were once lumped together, but are now considered to include different etiologies, bad breath cannot be considered a single entity. Even malodors stemming from various locales within the oral cavity smell different, and should be further distinguished based on molecular criteria.

Appendix 2

In Vitro Oral Malodor Assessment: Are Dentists Different?

Dan Markovich[1], Sarit Goldberg[1,2], Ilana Eli,[1] and
Mel Rosenberg[1,2]

Although most cases of bad breath originate in the mouth, dentists are sometimes unwilling or unable to provide assistance and advice. One possibility is that dental practitioners are relatively insensitive to bad breath. Since malodors are a part of dentists' daily life, the profession itself may select for people with varying degrees of hyposmia (D. Lancet, personal communication). Alternatively, dentists may become accustomed to the odors of their profession, just as shoemakers become adapted to the smell of leather, etc. The purpose of the present investigation was to compare the ability of dentists to score *in vitro* odors simulating bad breath with a non-professional group, as well as with a group of scientists involved in oral malodor research.

Odors resembling bad breath are difficult to reproduce in vitro. Even putrefied saliva has a unique type of odor which may not be characteristic of any of the various kinds of bad breath odors (Rosenberg and Leib, this volume). However, we have recently found that saliva from subjects with periodontal disease, following inoculation (1:25) in decarboxylase medium (DC) and anaerobic incubation, produces an odor akin to the subgingival plaque type of oral malodor (see Goldberg *et al.*, this volume). The resulting odor is modulated to some extent in the presence of added amino acids. Based

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D. Markovich, S. Goldberg, I. Eli and M. Rosenberg, The Maurice and Gabriela Goldschleger School of Dental Medicine[1] and Department of Human Microbiology[2], Sackler Faculty of Medicine, Tel Aviv University, Ramat-Aviv 69978,Tel Aviv, Israel

Table 1
Age and Gender Distribution

Group	N	Number of males	Mean Age ± SD (years)
Dentists	35	27	44.0 ± 12.9
Researchers	31	23	42.3 ± 9.9
Control	33	19	41.9 ± 16.7

Table 2
Mean VAS Scores

	Samples			
Groups	**Saliva + DC**	**Saliva +DC +tyrosine**	**Saliva +DC +lysine**	**DC alone**
Dentists	53.5[a]	42.8	37.9	18.6
Researchers	52.3	44.2	42.0	17.3
Control group	61.8	50.7	49.7	20.3

[a] Millimeters from 0 to 100 on the VAS.

on these observations, saliva was inoculated either into decarboxylase medium alone, or decarboxylase medium containing 0.5% tyrosine or 0.5% lysine. Cultures were incubated for 72 hours at 37°C, then centrifuged and filtered (0.22 μ pore size) to remove cells. Supernatants were maintained in tightly sealed vials for odor determination. Decarboxylase medium itself served as the negative control.

The following groups of individuals were asked to score the four samples: (i) 37 dentists; (ii) 33 scientists attending the First International Workshop on Oral Malodor ("researchers"); and (iii) 31 individuals who were neither

Table 3
Significance of Differences Between Means (Paired t-test)

	Saliva + DC +tyrosine	Saliva +DC +lysine	Saliva +DC
Saliva +DC +tyrosine		NS[a]	0.012
Saliva +DC+lysine			0.002
DC alone	<0.001	<0.001	<0.001

[a] Not significant

dentists nor malodor researchers (control). Age and gender distribution of the three groups are summarized in Table 1. Differences in gender and age distribution among the three groups were not significant.

Samples, in opaque glass vials, were presented to each subject in a randomized manner. Each subject smelled each bottle at will, and was allowed to go back and smell previous samples. Subjects first scored each bottle for resemblance to actual oral malodor, using a continuous 10 cm visual analog scale (VAS), ranging from no similarity whatsoever to identical odor. Subjects then scored the odors by rank, starting with the bottle approximating most closely the actual smell of bad breath, ending with that least resembling bad breath. Odor evaluations were conducted in well ventilated areas.

Results of the mean VAS scores are summarized in Table 2. In all three groups, saliva incubated in DC alone was given the highest mean score, followed by saliva incubated in DC with addition of tyrosine, and saliva incubated in DC with addition of lysine. As expected, the control vial (DC alone) received the lowest scores. No significant differences were found among the three subject groups (ANOVA). We then tested for differences among the four odor samples, taking the entire subject population (N=101) together. Results are shown in Table 3. Whereas the VAS scores of the two incubated samples containing DC with additional amino acids were not significantly different from one another, all other samples differed significantly.

Results of the ranking scores approximated those of the VAS. The saliva + DC sample was given first ranking by 58%, 57% and 40% of the dentists, researchers and control groups, respectively. DC medium alone was ranked last by 60%, 77% and 75% of the dentists, researchers and control groups, respectively. As with VAS, differences in ranking were not significant among the three subject groups.

The results of the present study do not support the hypothesis that dentists differ from other individuals in the manner in which they score samples resembling bad breath. Similarly, no significant differences were found in the ratings of the oral malodor researchers, as compared with the other two groups. Whereas the data do not rule out the possibility that differences do exist, they are probably subtle, and may depend on the context in which the measurement is made. For example, dentists may react differently to oral malodor when they perceive it in their daily practice.

Appendix 3

Current and Future Semiconductor-Based Bad Breath Detectors

Ken Yaegaki[1]

In the late 1980s, a Japanese dental products company (GC Dental Products Corp., Tokyo) developed two kinds of compact bad breath detectors for the dental and public market. Both detectors were equipped with a semiconductor sensor. Consequently, other Japanes companies have introduced a number of other bad breath detectors based similarly on semiconductor sensors (Tsunoda, 1988). Currently, the detectors marketed to the general public can be purchased in Japan by mail order or by telemarketing. There are, however, some problems with these detectors: In general, the scientific credentials of the detectors have yet to be established. Studies comparing the detector with gas chromatographic (GC) or organoleptic (odor judge) measurements have not been well documented. Furthermore, it has not been clearly stated whether the detectors are also sensitive to organic volatiles in mouth air other than methyl mercaptan or hydrogen sulfide.

Although specificity regarding volatile sulphur compounds (VSC) is an essential requirement for a bad breath detector, no detector in use except GC with a flame-photometric detector (GC/FPD) has enough specificity to analyze VSC. Since organic vapors do not cause deterioration of the semiconductor sensor, there is no need for it to be periodically replaced. Nevertheless, water vapor in mouth air may affect the sensor's sensitivity (Ehara, 1990). In comparing the analyses of human mouth air samples using

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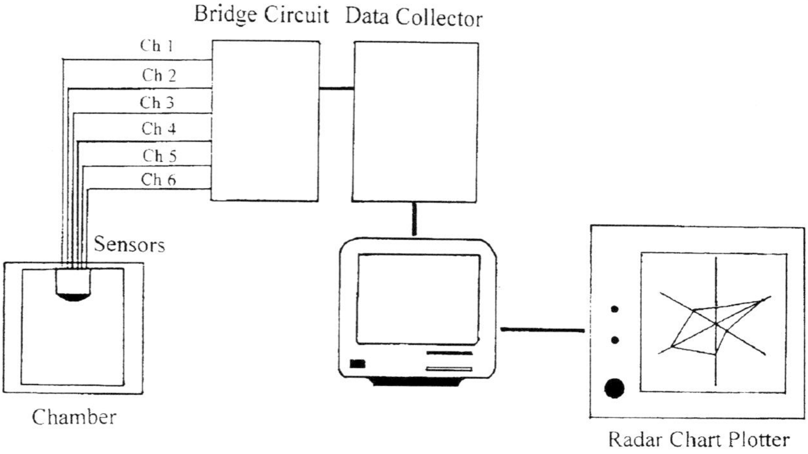


Figure 1.

commercial bad breath detectors, as compared with GC/FPD, I have observed that bad breath detectors have a low specificity for VSC. Some disagreements with the results of GC analysis were also noted. Occasionally the commercial detectors indicated "no malodor" or "no problem", yet the mouth air contained a high concentration of VSC as determined by GC/FPD (Yaegaki, unpublished data). As described in earlier chapters of this volume (Yaegaki, Rosenberg and Leib, Eli *et al.*) many halitosis patient have exaggerated concerns regarding their breath odor. Therefore, clinicians must be careful to first establish a rapport with their patients, as otherwise failure in completing the treatment may occur (Kanamori *et al.*, 1991). In order to prevent losing the patient's confidence, one should not use an unproven detector for diagnosis of halitosis. Of course, GC/FPD is the best objective method to diagnose halitosis, and can be compared closely to organoleptic measurement by a group of trained panels. Although patients, who may have psychosomatic tendencies, prefer instrumental objective measurement rather than the organoleptic evaluation, unfortunately GC may not be suitable for the dental practitioner because of its cost, complexity and expertise required to operate it.

Recently, New Cosmos Electric Co. Ltd. (Osaka, Japan) introduced a novel bad breath monitor which utilizes a zinc-oxide semiconductor sensor, similar to previous detectors, as do previous detectors. However, their monitor is

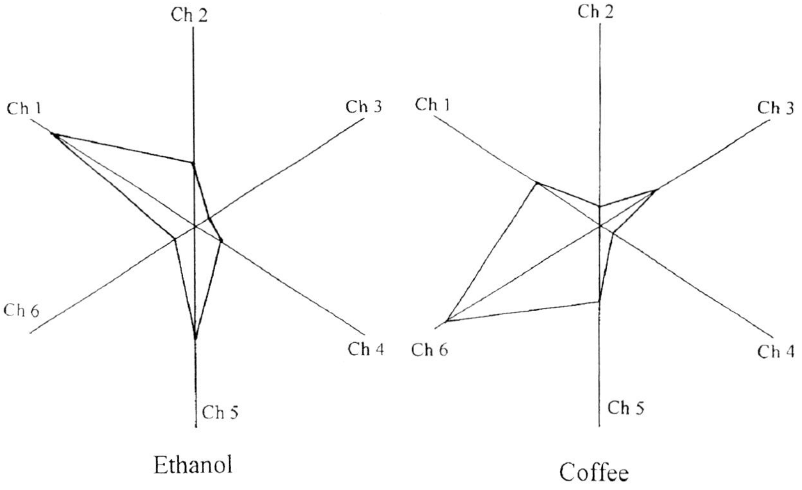
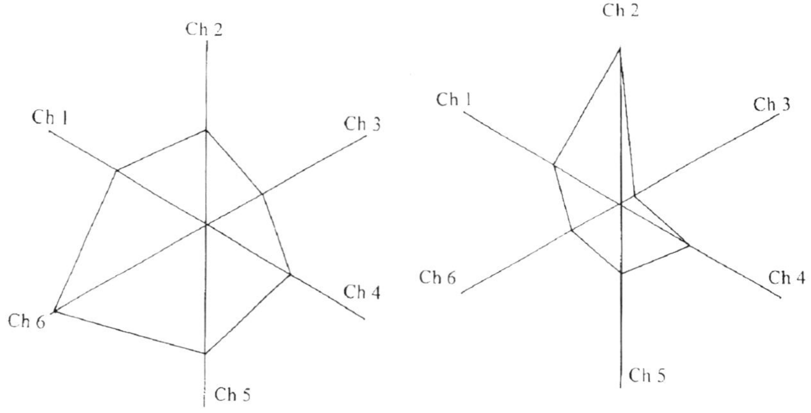


Figure 2.

equipped with a special filter which is intended to remove acetaldehyde, acetone and ethyl alcohol from mouth air samples, and thereby improve the specificity for VSC. If the monitor has a high specificity for VSC alone, this would yield a higher correlation between the monitor readings and VSC measurements conducted by GC. However, the correlation coefficient which they reported was still 0.75 (Shimura *et al.*, 1996). Therefore, it appears that the monitor may detect other volatile compounds along with the VSC, or that the semiconductor may not be reliable. Although this monitor appears to be much more accurate than previous detectors, it may still require further improvement of the filter system or development of a novel sensor superior to the zinc-oxide semiconductor.

Dr. Ehara and I have also developed a novel bad breath detector which has very high specificity for VSC. We modified the odor visualization method, which Dr. Ehara (1993) developed for industrial use. The system, which is shown in Fig. 1, demonstrates the specific properties in a radar chart plotting by six semiconductor sensors, each with a different specificity. Figure 2 shows the difference between odor properties of coffee and ethanol. Our system recognizes a good smell as a spearheaded shape in the radar chart, and bad smell as irregular shapes. Figure 3 shows the results of mouth air



GC analysis of mouth air containing:
Hydrogen sulfide 1.4 ng/10 ml
Methyl mercaptan 1.6 ng/10 ml

GC analysis of mouth air:
No detectable concentration of VSC
were found.

Figure 3.

analysis which are compared with data obtained using GC. The results indicate that the specificity of the system for VSC is much better than for other bad breath detectors. We found, however, that there were still some problems with the system: (i) it required greater specificity for VSC to diagnose halitosis; (ii) the system occasionally failed to recognize the differences in VSC composition; (iiii) water vapor in mouth air affected the sensitivity of the sensors. Therefore, our recent research focuses on solving these problems by improving the sensor, or by modifying the data processing. We are also searching for some revolutionary concept, which differs completely from conventional detectors. Recently, a novel diagnosis of gastric ulcers based on breath analysis was developed (Graham *et al.*, 1987). After the patient takes a nonradioactive tracer (^{13}C-urea), a metabolite in the patient's breath, which is produced by *Helicobacter pylori* associated with the gastric ulcer, is examined. Otsuka Pharmaceutical Co. Ltd. (Japan) has recently developed a compact device for detecting ^{13}C-metabolite in the patient's breath (E. Nonomura, personal communication). I am currently investigating the possibility of applying a similar approach to oral malodor research. In order to develop a bad breath detector which can serve as a real artificial nose, some novel and extraordinary concepts may be required.

The immediate objective is to develop a novel, compact and simple bad breath detector with high specificity for VSC, capable of distinguishing differences in VSC composition. If successful, I anticipate that my theory that the methyl mercaptan/hydrogen sulfide ratio increases in periodontal disease may serve as a useful parameter for periodontal screening in public dental health (Yaegaki and Sanada, 1992).

Acknowledgment

I thank Dr. J. Tonzetich, Professor Emeritus, and Dr. B. Chehroudi, Department of Oral Biology, The University of British Columbia, for their assistance in preparing this manuscript.

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Appendix 4

BDA Fact File

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Bad breath

July 1996

Bad breath (halitosis) is a common problem which often comes from the activity of bacteria in the mouth. Although there is no way of knowing for sure, most adults probably suffer from bad breath occasionally, with perhaps a quarter suffering on a regular basis. This fact file looks at the causes and at what dentists can do to help.

Is bad breath always treatable?
In the past, bad breath was often considered to be an incurable affliction. However, in recent years it has become increasingly evident that bad breath is usually treatable once a proper diagnosis is made.

The main problem is knowing whether we have it or not, because we are poor judges of our own breath odour. Some people suffer from bad breath without knowing it, while others build up exaggerated fears about breath odour even though they do not have it. The best way to find out whether we have bad breath is to ask for someone else's opinion. If we don't ask, other people are unlikely to tell us. And since bad breath can sometimes - fortunately rarely - be a sign of a significant general health problem, we should not be reluctant to tell people dear to us that they have a bad breath problem.

What should I do if I have bad breath?
If you have reason to believe that there is a problem, then see your dentist first, since bad breath often comes from the mouth itself.

When you see the dentist, it is a good idea to explain in advance that you will be asking for advice about bad breath. Also, try to go with someone who is familiar with the problem, to help give the dentist an objective picture of how bad the odour really is, how long it has been going on, and when it improves or gets worse. Since bad breath often varies, a family member or friend can also help determine whether the odour at the time of the appointment resembles, both in character and intensity, the odour that is generally troublesome.

If the dentist knows that the consultation is about bad breath, you may be asked not to eat, drink, smoke, chew gum, suck confectionery, use mouthwashes, breath fresheners etc., so that the odour will be more typical. You should also avoid using perfumed cosmetic products, such as perfume, aftershave and scented lipstick prior to the appointment, since it can interfere with the odour assessment. If the dentist is not told about the reason for the consultation beforehand, do these things anyway and tell the dentist that you have prepared for the appointment in this way.

What will happen at my appointment?
Your dentist will ask questions to help determine the possible causes of the odour, and then compare the odour coming from your mouth and nose. In most cases (about 85-95%) , the odour comes from the mouth rather than the nose. This is an indication that bacterial activity somewhere in the mouth is responsible. If the odour comes mostly from the nose, then the nasal passages may be involved.

Your dentist may also make measurements using a sulphide monitor to help in diagnosis and treatment, since volatile sulphur compounds are often associated with bad breath.

This table summarises different odour-related problems, and their possible causes.

Problem	Possible cause or source of malodour
Odour after fasting, dieting, sleeping, taking medications, prolonged speaking, exercise	dryness in the mouth, insufficient saliva flow
Gums bleed and/or smell	gum problems, poor cleaning between teeth
Odour upon talking	postnasal drip on back of tongue
Odour at onset of menstrual cycle	swelling of gums
Small whitish stones with foul odour appear on tongue	tonsilloliths from crypts in tonsils
Odour appears suddenly from mouth of young children	onset of throat infection
Odour appears suddenly from nose of young children	foreign body placed in nose
Odour appears suddenly from entire body of young children	foreign body placed in nose
Taste or smell of rotten fish	trimethylaminuria (rare)
Odour in denture wearers	dentures kept in mouth at night or not cleaned properly
Odour from nose	sinusitis, polyps, dryness, foreign body, hindered air or mucus flow
Bad taste all day long	poor oral hygiene, gum disease, excessive bacterial activity on tongue

Where does the odour come from?
Most cases of bad breath appear to be due to the breakdown of proteins by a variety of micro-organisms. Several of the breakdown products are foul smelling gases.

In people with healthy teeth and gums, the odour usually comes from the far back region of the tongue, and grows stronger when the patient starts talking. The dentist can sample this area using a plastic spoon. The odour coming from the spoon sample may then be compared to the overall odour. Although we do not know why, the very back of the tongue is an important source of bad breath, possibly as a result of postnasal drip, which can get stuck on the tongue and is then broken down by bacteria on the tongue surface.

If the back of the tongue is the problem, then the dentist can recommend a method of cleaning the area, either with a toothbrush, or a specially designed tongue scraper (in some countries, tongue cleaning is a common and ancient practice). It takes time and patience to overcome the gagging reflex. But, eventually, tongue cleaning becomes easy. Care should be taken to clean the back of the tongue thoroughly yet gently, without inflicting pain or sores.

Can gum disease cause bad breath?
In some people, bad breath is associated with gum disease, especially if rubbing the areas between the teeth and gums yields a foul odour. Your dentist can help prevent and treat gum diseases in various ways, depending on the type and extent of the problem, but your own daily home care makes all the difference in the world in maintaining gum health between appointments. Cleaning of the spaces between the teeth is of great importance. One home tip to healthy gums (and less bad breath) is to smell the odour coming from the dental floss, and to work to clean those areas more carefully. People with gum disease often have higher levels of odour coming from their tongue, as well.

What type of treatment is there?
Your dentist may recommend dental treatment, if there are other areas in which bacteria and food can become trapped and cause odour. The dentist may also suggest daily rinsing with one of several available mouthwashes which have been scientifically shown to reduce bad breath over time.

Your dentist may also refer you to clinics that specialise in identifying breath odours, or to other medical experts.

What can I do?
In all probability, professional diagnosis and treatment can help turn bad breath into good breath. However, it is sometimes difficult for us to sense the improvement ourselves. In this case, a family member or close friend can also provide important feedback and reinforcement.

Listed below are some of the Do's and Don'ts regarding bad breath. Remember, bad breath is a problem that needs professional attention. Don't mask it - deal with it.

Do's

- Visit your dentist regularly.
- Have your teeth cleaned periodically by a dental professional.
- Floss or otherwise clean between your teeth, as recommended by your dentist. Choose unscented floss so that you can detect those areas between your teeth that give off odours, and clean them more carefully.
- Brush your teeth and gums properly.
- Ask your dentist to recommend a toothbrush or scraper for your tongue. Clean your tongue all the way back gently, but thoroughly.
- Drink plenty of liquids.
- Chew sugar-free gum for a minute or two at a time, especially if your mouth feels dry. Chewing parsley, mint, cloves or fennel seeds may also help.
- Clean your mouth after eating or drinking milk products, fish and meat.
- Unless your dentist advises otherwise, soak dentures overnight in antiseptic solution.
- Get control over the problem. Ask a family member to tell you whenever you have bad breath.
- If someone in your family or a close friend has bad breath, find a kind way to let them know. If you can't tell them directly, leave this fact file lying around. They may get the message.
- Ask your dentist to recommend a mouthwash which has been shown to be clinically effective in fighting bad breath. Use it most effectively right before sleeping.
- Eat fresh, fibrous vegetables such as carrots.

Don'ts

- Don't let your concern about having bad breath run your life. Don't be passive.

- Don't be depressed. Get help. Don't ignore your gums - you can lose your teeth as well as smell bad.
- Don't drink too much coffee - it may make the situation worse.
- Don't forget to clean behind the back teeth in each row.
- Don't brush your tongue with regular toothpaste - it's better to dip your toothbrush in mouthwash for tongue cleaning.
- Don't run to the gastroenterologist for concerns of having bad breath - it usually comes from the mouth and almost never from the stomach.
- Don't give mouthwash to very young children, as they can swallow it.
- Don't clean your tongue so hard that it hurts.
- Don't rely on mouthwash alone - practise complete oral hygiene

This fact file has been prepared by Professor Mel Rosenberg, of Tel Aviv University, Israel with help from Dr Stephen Porter and Dr Philip Stemmer.

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Index

Accompanying persons, 144
Acetic, 34, 35,160, 163, 168
Acetone, 151
Actinomyces naeslundii, 29, 57
Actinomyces viscosus, 29
Active oxygen, 92, 95
Acute necrotizing ulcerative periodontitis, *see also* Periodontal diseases, 110
Adaptation, 2
Additivity, 216
Adult periodontitis, *see also* Periodontal diseases, 10
Advertisements, 144
Alcohol, 221
Aliphatic acids, volatile, 151, 163, 168
Alkalinity, 27, 71
Allergies, 139
Alpha-ionone, *see* Ionone
d-Aminovaleric acid, 73
Ammonia, 73
Amoxycillin, 194
Anaerobic lung abscess, 175
Anaerobisis, 71
Anaphylactia, 90
Aniline, 151, 158
Antibacterial, 62-65, 133
Anxiety, 201, 205-211
Arginine, 13, 22, 24, 27, 74, 77, 78, 83
Aromatic amines, 152
Asaccharolytic bacteria, 44, 48, 49, 52
Asparagine, 27
Augmentin, 194

Bacteria, assacharolytic, 44, 48, 49, 52
Bacterial endotoxins, xv
Bacterial load, 52
Bacterial metabolism, 35, 109, 168
Bacteroides, 21, 22, 47, 71, 181, 184
Bacteroides forsythus, 49
Bacteroides gingivalis , *see Porphyromonas gingivalis*
Bad breath,
    control, 62
Bad taste, *see* Taste, bad
BANA test, 3, 4, 41, 48, 49, 52, 75, 169, 217, 113
Basal membrane, xiv, 8-91
Basic pH, *see* Alkalinity
Belching, 146
Benzethonium chloride (BTC), 62, 64
Benzoyl-DL-arginine naphthylamide, *see* BANA test
Beta-ionone, *see* Ionone
Black-pigmented bacteroides, 44
Bleeding index, 94, 97, 100
Blood, 56, 100-102, 161, 162
Body image, 202, 203, 221

Body odor, *see* Odor, body
Brushing, 129
Butyrate, 34, 35, 43, 44, 74, 96, 160

Cadaverine, xvii, 3, 4, 14, 34, 35, 73-83, 217, 222
Caldwell-Luc operation, 195
Calicified object, *see also* Foreign body, 140
cAMP, xv
Candida albicans, 57
Capnocytophaga, 48
Carbohydrate, 27, 3, 51, 72
Carbon monoxide, 10
Casein, 97
Caseous tonsillitis, *see also* Tonsils, 183
Caucasians, 156
Centipeda periodontii, 47
Cephalexin monohydrate, 194
Cetylpyridium chloride (CPC), 62
CH_3SH, *see* Methyl mercaptan
Chair side, *see also* Measurement, 169
Chemiluminescence detector (SCD), xiii
Chemosensory Clinical Research Center, 154
Chewing gum, 222
Children's bead, *see also* Foreign bodies, 140
Chlorhexidene, 9, 49, 52, 53, 90, 115, 116, 170, 218
Choline, 155, 157
Chronic ethmoiditis, 176
Chronic mucosal disease, 179
Chronic tonsillitis *see* Tonsillitis, chronic
Cigarettes, *see also* Smoking, 221
    odor of, 140
Cirrhosis, xii, xvii, 168
    decompensated, 151
Clavulanic acid, 194
Cleft palate, 8, 189, 190, 192, 196, 198, 199, 219
Clinical diagnosis, 110, 175, 182
CO_2 laser, 183, 184, 186, 127, 130, 131, 132, 134, 170, 218, 219
Coffee, 220
Collagen, xiv, xv, xvii, 87, 89, 91, 95, 152
    fibers, xiv, 91, 92, 93
Colorimetric indicators, 169
Community Periodontal Index of Treatment Needs (CPITN), *see also* Periodontal diseases, 119, 121
Computerized tomography (CT), 178-182
Concha bullosa, 179, 180-82
Connective tissue, xiv, xv, xvi, 89, 90
Consultation services, *see also* Counseling, 137, 154
Contact point, 43, 51
Cotton wool applicator, 34
Counseling, 9, 10, 105
Craniofacial anomalies, 8, 189, 197-199, 219

Craniomandibular pain patients, 204
Cresol, 72, 73
Crevicular fluid, xv, 27, 41, 43, 57-59, 72, 216
CT, *see* Computerized tomography
Cystathionuria, 158, 160
Cysteine, 13, 22, 24, 41, 44, 45, 47, 50, 56, 58, 72, 88, 95-98

Debridement, 42, 52, 53, 109, 170,182, 183
Decarboxylase medium, 6, 74-83, 223, 224
Decarboxylase, 6, 74-79, 83, 224
Deep pockets, *see also* Periodontal diseases, 75, 80, 81, 83
Delusion, *see also* Imaginary bad breath, 9, 145, 203, 205, 211
Dental phobics, *see* Phobics, dental
Dental plaque, *see* Plaque, dental
Dentifrice, 61, 65
Dentists, 142, 152, 199, 223, 226
Dentures,
  odor, 140
  unclean, 202
Depression, 201, 204-206, 208-211
Diabetes mellitus, xii, 151
Diagnostic tests, *see also* Measurement, 110, 111
Diamines, 74-79, 83, 204, 217
Dimethylamine, 151
Dimethyldisulfide (DMDS), 158-160
Dimethylsulfide (DMS), 73, 150, 151, 159, 160
Diphenylamine, 73
Divorce, 1
Dodecanol, 72, 73
Dry mouth, *see also* Saliva, 202
Dysgeusia, 154, 156, 168
Dysosmia, 154, 156, 168

Early detection, 7, 110, 111
Eating habits, 51
Ectopic tooth, 197-199
E_h, *see* Oxidation-reduction potential
Empyema, 175
Endoscope, 177, 180
Endotoxin, 89, 93, 95
Epidemiological survey, 121
Epithelial
  cells, 19, 20, 72, 102, 130
  squames, 13, 14, 20
Esophagus, 146
Ethanethiol, 151
Ethmoidal labyrinth, 176
Eubacterium, 44
Eubacterium limosum, 47
Eugenol, 64
Exfoliated cells, 72

Faulty restorations, 202
Fecal odor, *see* Odor, fecal
Fetor hepaticus, *see also* Cirrhosis, 151

Fibroblast, xiv, xv, xviii, 91
First International Workshop on
    Oral Malodor, 224
Fishy, *see* Trimethylaminuria
Fishy odor, *see* Odor, fishy
Fishy taste, 158
Flame-photometric detector
    (FPD), see *also*
    Measurement, xiii, 155
Fluoride, 65, 66
Food impaction, 202
Foodstuffs, 221
Foreign bodies, 140,199
    frequency of, 217
Fusiforms, 48, 71
Fusobacterium nucleatum, 44, 48,
    56, 57, 92, 181
Fusobacterium polymorphum, 48

G-protein, 220
Gagging reflex, 146
Garlic, *see* Pizza
Gas chromatograph, xii, 3,17, 55,
    58, 59, 72, 88, 94, 102-104,
    111, 150, 155, 161, 169, 216,
    219
Gas sensors, *see also*
    Measurement, 103
Gastrointestinal tract, 146
Gastrointestinal ulcers, xii
Gastroscopy, 146
Gauze pad, 146, 191, 192, 194, 219
Genetic factors, 220
Gingival crevice, xii, 15, 56, 131
Gingival crevicular fluid (GCF),
    see *also* Periodontal
    Diseases, 27, 41, 43-45, 50,
    51, 57-59, 87, 88, 97-99, 216
Gingival Index *see also*
    Periodontal diseases, 57, 58,
    75, 113-115, 218, 219
Gingival inflammation, 58, 67
Gingival recession, *see also*
    Periodontal diseases, 110
Gingivitis, *see also* Periodontal
    diseases, 9, 29, 43, 55-58,
    131, 216
Glossodynia, 205, 209, 211
Glucose, 14-19, 32, 33, 72, 217
Glutamine, 27
Glycolysis, 74
Glycoproteins, xviii, 50, 72
Gold standard *see also*
    Measurement, 2,112, 116

H_2S, *see* Hydrogen sulfide
Haemophilus, 13, 22, 32, 217
Haemophilus influenzae, 181
Haemophilus parainfluenzae, 29
Halimeter, *see also* Portable
    sulfide monitor, xiii, xvii,
    17-19, 103-105, 169, 216,
    217, 219
Halitophobia, *see also*
    Imaginary bad breath, 9,
    105, 147, 209
Headspace, xii, xiii, 17-19, 58,
    72-74, 78, 83, 150, 152, 155,
    203
Hemin, 101
Hemoglobin, 101
Hepatic cirrhosis, *see also*

Cirrhosis, xii, xvii
Hexadecanol, 72, 73
Hiatus hernia, xii
Hippocrates, 88, 109, 149
Histidine, 24, 77, 78
Hormonal changes, *see also* Menstrual cycle, 57
Hostility, 201, 205-211
Hydrogen peroxide, 32
Hydrogen sulfide, xiii, xiv, 4, 7, 10, 14, 17, 19, 24, 32, 34, 35, 43-48, 55-59, 64, 67, 71-73, 77, 78, 87, 88-104,115, 116, 129, 150-168, 216, 221
Hydroxyproline, 91
Hypochondria, 9
Hyposmia, 223

Imaginary bad breath, 9, 145, 147, 219
Immotile cilia syndrome, 179
Indole, 3, 14, 34, 35, 71-74,158, 159, 222
Industrial sulfide monitor, *see* Portable sulphide monitor
Initial preparation, *see* Periodontal diseases
Instrumental methods, *see* Measurement
Integrin, xviii
Internal hemorrhage, xii
Interpersonal sensitivity, 201, 204-209
Ionone, 66
Islamic teachings, 1
Isobutyric, 160
Isovaleric, 160

Juvenile periodontitis, *see* Periodontal diseases

2-Ketobutyrate, 87, 96, 99
Ketones, 151
Ketonic breath, 151
Ketuba, 1
Kidney failure, 151
Klebsiella pneumoniae, 56, 56-58
Kleinfelter Syndrome, 140

Laboratory research, 137
Lactic acid, 71, 73
Lactobacillus acidophilus, 57
Larynx, 175, 177
Laser cryptolysis, *see also* Tonsilloliths, 147, 183, 184, 186, 187
Lead acetate, 57
Leukemia, xii
Leuven, 8
Limonene, 151
Listerine, 9
Liver Disease *see also* cirrhosis, 151
Loose teeth, 110
Low salivary flow, 158, 159, 160
Lower respiratory tract, 175
Lung air, 155, 158-165, 168
Lung carcinoma, 151
Lysine, 77, 78, 83, 224, 225

Magnesium, 63
Marriage, 1, 2
Masking, xiii, 9, 42, 62, 216
Mass spectrometry *see also* Measurement, 150, 161
Measurement, xiii, xvii, 3, 4, 17, 18, 34, 47-52, 55, 59, 72, 78, 79, 112-116, 130, 150
  instrumental methods, 55, 59, 61, 62, 67, 68, 150, 169
  organoleptic, 6, 19, 51, 59, 76, 78, 155
Meat, 43, 180
Medical breath testing, 10
Medical history, 137
Menstrual cycle, 7, 150
Mental retardation, 140
2-Mercaptoethanol, xv
Meta analysis, 220
Metallic taste, 158
Methionine, 13, 22, 24, 41, 44, 45, 47, 50, 72, 87, 95-99
Methyl mercaptan, xii, xiii, xiv, xv, xvii, xviii, 7, 14, 17, 34, 35, 41-48, 55, 64, 72, 73, 87-104
Methylamine, 3, 73, 154, 155, 157, 161, 165, 219, 222
Methylene blue, 29, 31
Methylethylketone, 151
Methylpyridines, 72, 73, 152
Metronidazole 77, 78, 83, 184
Microbiota, *see* Microorganisms,
Microcephalus, 140
Microorganisms, 4,16, 25-29, 41-53, 55, 56, 83,112, 177, 184, 197, 217
  Gram-negative,13, 21, 23, 27, 30, 31, 35, 41, 50, 55, 56, 59, 62, 67, 71, 83, 93, 216
  Gram-positive, 13, 21, 23, 27, 30, 55, 56, 72, 181
Micros prevotii, 47
Middle turbinate, 178, 179, 180
Mint, 216
Monitor *see* portable sulphide monitor
Monooxygenase, 156
Moraxella catarrhalis, 181
Mouth, 202-206, 209, 211, 212
Mouthrinsing, 9, 143
Mouthwash, 9, 90, 91, 105, 170, 199
  two-phase oil-water, 9, 53, 169
Mucins, 27
Mucociliary clearance, 176, 179, 182
Muhamed, prophet, 1
Multicenter study, 220
Multiple regression analysis, 75, 76, 82
  stepwise, 115

Nasal malodor, 8, 115, 139, 140, 143, 158, 218
Nasal passages, 8, 147, 177, 190
National Institute of Deafness and Other Communicative Disorders, 154
Necrotizing pneumonia, 175
Neisseria, 13, 21, 32

Neutral proteases, xv
Nutrient supplies, 43

Obsession-compulsion, 9, 201, 204-209
Odor,
  body, 10, 154, 141, 154, 158-160, 202, 203, 211
  fecal, 158
  fishy, 151
  skunk, 66
  smokers' breath, 140
  sour-type, xvii
  visualization method, 104
Odor judge, 3, 6, 75-77, 138-147, 217, 218
Olfactory reference syndrome, 9, 203
Olfactory sub-genome, 220
Onion, *see* Pizza
Oral hygiene, 9, 29, 43, 52, 61, 105, 121, 137, 143, 146, 175, 202, 216
Oral malodor control, 55
Oral wetness, 114
  oralis, 25, 26, 28
Organoleptic, *see* Measurement
  organoleptic method, 61, 67
Ornithine, 13, 22-25, 74, 77, 78, 83, 217
Oronasal fistulas, 199
Oropharyngeal, 151
Osler, Sir William,109
Osmoscope *see also* measurement), xi, 3
Osteomeatal complex, 176, 182
Otolaryngologic examination, 182
Otolaryngologist, 140, 164, 175, 176, 180, 190, 193, 197
Oxidation-reduction potential, 14, 17, 29, 31, 32, 33, 35
N-oxidation, 156, 157
Oxygen depletion, 29, 32

$P0_2$ *see* Oxidation-reduction potential
Paper points, 74, 79, 81, 82
Paper strip, 34, 53, 57
Paraffin wax, 14
  parainfluenzae, 29
Paranasal sinuses, 176, 179, 181
Paranoid ideation, 201, 205-209
Patient throughput, 111
Pediatric patients, 199
Peptides, 14, 15, 17, 20, 27, 41, 42-44, 51
Peptococcus, 44, 47
Peptostreptococcus anaerobius, 47
Perceived bad breath, *see* Imaginary bad breath
Perception, xvii, 53, 142, 166, 201-204, 207, 220
Periodontal diseases, xii, xiv, xv, xviii, 7, 22, 29, 31, 36, 41, 43-48, 51, 52, 57, 58, 72, 77-79, 82, 83, 87-89, 93-98, 100-102,109-116, 120, 130-132, 150, 152, 160, 202, 218-223
  acute necrotizing ulcerative

  - periodontitis, 110
  - Community Periodontal Index of Treatment Needs (CPITN), 119, 121
  - initial preparation, 219
  - juvenile periodontitis, 110
  - periodontitis, 109, 113
  - prepubertal, 110
- Periotron, 33, 34, 58,114
- Pesticides, 166
- pH, xv,14, 16-18, 20, 21, 25, 27-29, 32, 35, 56, 58,72, 74, 77-80, 83, 115, 155, 217, 218
- Pharyngeal flap surgery, 190, 191
- Phenol, 72, 73
- Phenolic flavor oils, 63-65
- Phenylethyl alcohol, 165
- Phobic anxiety, 201, 205-209
- O-phthaldialdehyde, 75
- Picoline *see* Methylpyridines
- Pizza, 9, 220, 222
- Plaque, 7, 9, 15-17, 20, 22, 23, 27-29, 31, 32, 41-46, 50, 56, 74, 75, 114, 119-121, 161, 169, 203, 217-219, 223
- Plastic spoon, *see also* Measurement, 138, 139, 146, 147
- PMA index, *see also* Periodontal diseases, 119
- Pneumonitis, 175
- Pockets, *see also* Periodontal diseases, 31, 46, 71, 74, 79-83, 88, 89, 92, 94, 98-100, 103, 113-115, 130, 132, 218
- Polyamines, 42
- Polymorphonuclear leukocytes, 92
- Polypoid, 179, 182
- Polyposis, 179
- *Porphyromonas endodontalis*, 46
- *Porphyromonas gingivalis*, 41, 75, 93, 101, 217
- Portable sulfide monitor, *see also* Halimeter, xii, 3, 72, 75, 78, 111, 119, 120, 216-218
- Postnasal drip, 8, 146, 147, 176, 177, 180, 181, 190
- Potassium tellurite, 77, 78, 83
- Preconception *see also* Imaginary bad breath, 139, 204, 206, 211
- *Prevotella intermedia*, 44, 217
- *Prevotella melaninogenica*, 50, 56-58
- Primary biliary cirrhosis, 151
- Probing depth, 75, 95, 96, 98, 104, 116, 203
- Proline, xiv, 91, 95
- n-Propanol, 151
- Prophylaxis, 57, 160
- Propionic acid, 34, 35, 43, 44, 160, 168
- Prostoglandin E_2 (PGE_2), xiv, xv, 89, 95
- Protein, xv, xvii, 14, 15, 17, 20, 27, 41-44, 51, 55, 71, 72, 87, 89, 90, 95, 97, 99, 100
- Proteoglycan, 89, 90
- Proteolytic activity, 42, 82
- Psychological counsel, 144
- Psychopathology, 201, 204

Psychoticism, 201, 205-209
Purulent discharge, 141, 179, 180, 193, 194, 197
Putrefaction, xvi, xvii, 4, 14, 15, 17, 35, 71, 109, 112, 146, 217, 218
Putrefied paper tissue, 141
Putrescine *see also* Diamines, xvii, 14, 34, 35, 73-83
Pyridine, 72, 73, 152, 165, 222
Pyridinoline, 152

Ramadan, 1
Rectum, 203
Redox dye, *see also* Oxidation-reduction potential), 29
Regeneration, 110, 111
Respiratory tract conditions, 175
Rhinoplasty, 8
Rhinorrhea, purulent, 196, 199
Rhinoscopy, 177, 180
Rothia dentocariosa, 48

Saccharolytic bacteria, 44, 48
Saliva, xii, xvi, xvii, 2-4, 13-20, 27, 29, 32, 33, 35, 36, 41-44, 50-52, 55, 56, 58, 71-83, 87, 96-102, 105, 131, 132, 143, 150, 152, 154, 155, 157-162, 181, 190, 204, 206, 211, 217-219, 223-226
  low flow rate, 51, 52, 153, 158-160
  putrefied, 76
  salivary glycoproteins, 27
  salivary sediment, 13-21, 25, 26, 32, 33, 35
  salivary supernatant, 14, 17, 18, 20, 25, 32, 33
Scales, odor measurement, 3, 5, 17, 21, 34, 60, 114, 139, 155, 204-206, 210
SCL-90 questionnaire, 201-207, 221
Sediment model, *see also* saliva, 16, 26
Selenomonad artemidis, 47
Selenomonas, 44
Self-esteem, 142
Self-estimation, 2,120, 128, 131, 138, 143, 144, 147, 202
Self-measurement, 2,143
Semiconductor, gas sensors, *see also* Measurement, 103
Sensitive teeth, 110
Serine, 27
Serum proteins, 41, 44-45, 45, 48-50
Shallow pockets, *see* Periodontal diseases, 80, 83
Shoemakers, 223
Sialopaper *see also* Periotron, 33, 34
Sinus, 8, 175-182, 189-198, 219
Sinusal ostia, 179
Siwak, 1
Skatole, 14, 34, 35, 72-74
Skin, 203
Skunk odor, 66
Smoking, 9, 42, 119-121, 120, 121, 128, 133, 135
Sodium bicarbonate, 63, 65, 66

Soft palate, 168, 197
Somatization, 201, 204-209
Spirochetes, 47, 71
Squamous cells, 99
Staphylococci, 181
Staphylococcus aureus, 57
Staphylococcus epidermidis, 29
Storage loop, 60
Streptococci, 181
Streptococcus mutans, 57
Streptococcus oralis, 25, 26, 28
Streptococcus pneumoniae, 181
Streptococcus salivarius, 57
Streptococcus sanguis II, *see* *Streptococcus oralis*
Streptococcus sanguis, 57
Subgingival putrefaction, *see also* Periodontal diseases, 139
Subject population, 7
Suicide, 9, 105, 145
Sulfide monitor, *see* Portable sulfide monitor
Sulfur-containing drugs, 166
SwiftLase®, 186
Systemic disease, 8, 109, 151

Taste
    bad, 144, 146, 150, 152, 154, 156-161, 168
Taurine, 98, 99
Teflon, 60
Tetradecanol, 72, 73
Thiol-tissue complex, 89
Threshold, *see also*
    Measurement, xvii, 88, 114, 155, 165, 166, 168, 216
Throat infection, 202
Thymol, 64
TMAU, *see* Trimethylaminuria
o-Toluidine, 151
Tomato juice, 66
Tongue, xii, 8, 23, 33, 41-53, 75, 76, 87, 100, 102-105, 113, 115, 121, 129, 130, 138-140, 142, 143, 146, 160, 161, 168, 169, 175-177, 180, 181, 190, 197, 198, 202, 204, 206, 211, 217-219, 221
    brushing, 9, 146
    cleaning, 105,
    coating, 46, 48, 87, 88, 102-105, 115, 116, 119-121, 129, 130, 140
    dorsum, posterior, xii, 7, 8, 75, 138-140, 146, 168, 169,
    odor, 140
    scraper, 102, 147
Tonsillitis, chronic, 175, 182, 183
Tonsilloliths, 144, 147, 183
Tonsils, xiv, xv, 8, 47, 144, 146, 147, 175, 177, 182-187
Tooth loss, 110, 111
Treponema denticola, 41
Trimethylamine, 151, 154, 155
Trimethylaminuria, 8, 150, 151, 154-168, 219
Triphenyltetrazolium chloride, 29

Trypsin-like activity, *see also* BANA test, 75, 113, 203
Tryptophan, 13, 22, 24, 25, 217
Two-phase oil-water mouthwash, *see* Mouthwash
Tyrosine, 224, 225

Upper Respiratory, 151
Urea, 27, 73
Uremia, xii, 151
Ureolytic activity, 29
Urine, 155, 157, 161, 162

Vagina, 203
Valeric acid, 43, 44
Variability, 112
Variations, 7, 113
Veillonella alcalescens, 56-58
Vibrios, 71
Visual analog scale (VAS), 75, 225
Vitamin K, 101
Volatile sulfur compounds (VSC), xii-xiv, xvi, xvii, 3, 17, 18, 41-52, 55-68, 71, 72, 75-78, 83, 87-97, 101-105, 110-116, 120-135, 149-153, 155, 158-163, 165-170, 190, 218
Vomerine spurs, 190

Wooden stick, 52
Wrist lick test, *see also* Tongue, 33

Xantopren, 195, 196

Yellowish discharge, *see also* Postnasal drip, 146

Zinc, *see also* Mouthwash, 63-68, 90, 91, 93, 105

ISBN 965-274-173-6